EXPERIMENTAL STUDIES ON TILLERING IN BARLEY

by

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SUMMARY

There were two main aims in this experimental project on tillering in barley; first, to obtain information on the rates of growth, and various aspects of stem development on both tillers and mainstem, and secondly, to study the early growth of tillers prior to their emergence above their subtending leaf sheaths, and the effects of various treatments on this.

Shading either the first or second mainstem leaf was found to reduce the numbers of tillers emerging and to decrease total plant grain yield without affecting yield of either the mainstem or individual tillers developing to maturity; the major effect of shading either leaf was shown to be on tiller development during early plant growth. Rates of leaf appearance on the mainstem and tillers developing to maturity were not affected by shading, and were found to be in the order - mainstem > primary tillers > secondary tillers. A similar order for the different stems was found when rates of primordial initiation were studied. These results indicate a hierarchical relationship between stems in the barley plant.

Both the onset and the rate of exponential increase in dry weight were also affected when the initial application of the complete mineral nutrient solution was delayed beyond day 2 after planting; there were also considerable effects of delaying the second application of nutrient. Withholding either nitrogen or non-nitrogenous nutrients, and lowering the amounts of nitrogen applied seriously affected tiller bud growth, and it was shown/

shown that the effects of lack of non-nitrogenous nutri-:ents were due primarily to the lack of phosphorus. TC was affected to a greater extent than T1 when nutrient application was delayed, but the reverse situation was found when amounts of nutrient applied were decreased. Tillers were consistently affected to a greater extent than the plant by delaying the application or decreasing the amounts of nutrients applied, indicating an effect of nutrient application on apical dominance.

Studies of tiller bud development in control condi-:tions were also carried out; both initiation of floral primordia, and awn appearance took place later on the tillers than on the mainstem, and both these developmental stages were found to be more or less synchronous on tillers T1 - T3. Early in plant growth the mainstem apical dome was larger than the corresponding region on any of the tiller apices, although after floral initiation apical dome size was similar on both mainstem and tillers T1 and T2. Relationships between young buds and the mainstem were investigated by both a ^{14}C-labelling expe-:riment, and an anatomical study of sectioned material from seedlings up to 10 days old.

From the results of the project possible routes for the passage of assimilates and mineral nutrients into tiller buds during their early development are suggested, and the implications of the results on current theories of apical dominance, and the possible importance of the commercial use of uniculm cereal varieties discussed.

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CHAPTER 1

INTRODUCTION

I GENERAL INTRODUCTION

Axillary buds in grasses may develop as stolons, rhizomes or as tillers. In the case of barley, Hordeum vulgare, all the buds are apogeotropic, growing upwards, and eventually emerging above the leaf sheaths; rhizomes and stolons are diageotropic, breaking through the base of the subtending leaf sheaths, as for instance in Agrostis stolonifera and Phalaris arundinacea (Jewiss, 1966).

In pasture grasses tillering is important in the establishment of the sward, and in regeneration after cutting or grazing (Jewiss, 1972), while in cereals its main significance is in increasing the number of stems potentially capable of yielding grain and thereby allow-:ing maximum utilisation of available resources of miner-:al nutrients and incident light. In addition to the economic importance of tillering it is also of great significance in relation to studies on apical dominance in monocotyledonous plants, and for these reasons much research has been carried out.

The tiller bud primordium in perennial grasses de-:velops as a result of cell divisions in the sub-hypo-:dermal layers of the apex (Jewiss, 1972), about 2-3 plastochrons after the initiation of the subtending leaf. Sharman (1945) has described the histology of tiller initiation, and the early relationship between mainstem and/

and tiller in Agropyron repens, and a description of leaf initiation and adventitious root production during vegetative growth is given by Jewiss (1966). Initially the tiller is dependent upon the mainstem for its supplies of assimilates, and Quinlan and Sagar (1962) have shown a movement of ^{14}C assimilate from the mainstem to the tiller in wheat. After the tiller has produced adventitious roots, and its leaves have developed as assimilating organs it is, to a large extent, independent of the mainstem, and can therefore be in competition with it for nutrients (Bunting and Drennan, 1966). However, vascular connection is maintained between a tiller and the mainstem throughout plant growth, and it is possible for the tillers to supply the mainstem with materials, as shown in maize (Dungan, 1931), and in wheat (Clifford, Marshall and Sagar, 1973), especially late in the life cycle when some tillers may become senescent. Gifford and Marshall (1973) have shown that in Lolium temulentum the mainstem can supply defoliated tillers with assimilates, even late in development; from this and similar evidence Marshall and Sagar (1965, 1968) have argued that the plant is an integrated unit, and that even after development to a stage at which the tillers are, in effect, independent of the mainstem, defoliation of the tillers results in re-integration of the plant. A similar situation is proposed in oats (Labanauskas and Dungan, 1956) and in wheat (Rawson and Hofstra, 1969), but in the pasture grass timothy (Phleum pratense) St. Pierre and Wright (1972) have suggested that each stem is an independent unit,/

unit, and that tiller defoliation does not result in re-integration of the plant.

With reference to the economic importance of tille-:ring in cereals there is some debate as to whether or not tillering is advantageous for maximum yield. Some workers (Fiddian, 1967; Donald, 1968) suggest that 'uniculm', non-tillering, varieties sown at a high density should theoretically produce more uniform crop growth, maturing over a shorter period of time, than conventional tillering varieties sown at a lower density. However, since uniculm varieties are not in commercial use at present, and tillering will always be of importance in pasture grasses, further research on the mechanism and control of tillering is required.

II FACTORS KNOWN TO AFFECT TILLERING

Work on tillering has been reviewed by Gardner (1942), Langer (1963) and Evans, Wardlaw and Williams (1964), and various aspects of tiller development are discussed in 'The growth of cereals and grasses', the Proceedings of the 12th Easter School in Agricultural Science, University of Nottingham (eds. Milthorpe and Ivins, 1966). Among factors known to affect tillering in grasses are the following:-

(i) Seed quality

Engledow and Wadham (1924) showed an effect of grain size on tillering, with larger grains producing plants tillering more extensively than those from small ones. The same authors quote Beavan as stating that a higher grain nitrogen content favours tillering. However, Dobben (1966)/

(1966) has given evidence that similar yields are obtained from plants developing from grain of both high and low nitrogen contents, provided that there are no adverse conditions of growth, such as severe competition from weeds.

(ii) Soil moisture

Maximal plant growth requires adequate water supply, and therefore it is to be expected that water stress re-:duces tillering. At different stages in the life cycle of a plant water stress affects growth in various ways (Slavik, 1966), and it is especially in young plants that water stress reduces tillering. Gardner (1942) experi-:menting on Marquis wheat, showed that tillering was greater in a 50% saturated than in 25% saturated sand quartz culture. Work by Husain and Aspinall (1970) has shown that apical growth in barley is seriously affected by water stress, and that leaf and tiller primordial initiation is reduced.

(iii) Mineral nutrition

Evans, Wardlaw and Williams (1964) state that nitro-:gen, magnesium, phosphorus, potassium and calcium have all been shown to affect tillering in cereals, and from this and other literature it is clear that supply of min-:eral nutrients has a very considerable effect on tillering.

Variation in the timing of nutrient application can alter the normal pattern of tillering, in which the appearance of new stems usually ceases when mainstem elongation begins to occur. Using Piroline barley Aspinall/

Aspinall (1961) showed that if all the nutrient were applied pre-germination there was a distinct two-phased pattern of tiller appearance. At a lower level of nutri-:ent application tillering ceased sooner, and after fewer tillers had been produced than when the full amounts of mineral nutrients were applied. A different pattern of tillering resulted from nutrient application at weekly intervals throughout the growth of the plant, and even a low, 5%, level of nutrient application supplied weekly resulted in continuous tillering compared with the two-:phased pattern produced with the standard nutrient solu-:tion applied pre-germination. Plants grown on a 5% nutrient solution applied weekly for 20 weeks produced approximately twice as many tillers as those supplied with the standard amount pre-germination, although the same total amount of nutrient was applied in both cases.

Using Plumage Archer barley Gregory and Sen (1937) showed that a tenfold increase in tiller number occurred when nitrogen supply was increased from 15 to 1200 mg nitrogen per pot. Gregory (1937) reported work showing considerable effects of phosphorus and potassium as well as of nitrogen on tillering; in plants supplied with either 56mg phosphorus or 167mg nitrogen per plant there was an increase in tillering as the supply of potassium was decreased until this was severely reduced to 1.3mg per plant. When the supply of either phosphorus or nitro-:gen was reduced to about 6mg per plant the effects of potassium deficiency were masked. From these and other experiments/

experiments Gregory concluded that in the control of tillering nitrogen has a greater effect than phosphorus, which in turn has a greater effect than potassium.

Thorne (1962a) applied nitrogen to barley either early in spring or at ear emergence, and showed that the former treatment resulted in an increased final ear number, and grain size, whereas the latter led to the production of new shoots which did not develop to maturity.

Sandfaer (1953) has shown that of the three components mainly responsible for yield in cereal crops, namely, ear number per unit area, number of grains per ear, and grain size, the latter two are to a large extent independent of nutrition unless it is reduced to starvation level, whereas ear number per unit area, that is, the number of tillers produced less the number of tillers which senesce, is greatly affected by nutrition.

(iv) Light intensity

Increasing light intensity resulted in an increase in the rate of leaf and floral primordial production in barley (Aspinall and Paleg, 1963), and Friend (1965) found that increasing light intensity increased the rates of both tiller and leaf appearance in wheat. At low light intensities only primary tiller buds developed (Aspinall and Paleg, 1964), whereas with higher light intensities secondary and higher order buds were also produced. Mitchell (1953a) found that at low light intensities tiller development from the basal nodes of the mainstem was inhibited to a greater extent than that from the/

the higher nodes.

(v) Photoperiod

There is a number of complicating factors regarding work carried out on the effect of photoperiod on tillering. Firstly, total incident radiation reaching the plant may have been altered by changing the photoperiod, so that amounts of assimilate in the plant could have been altered. Secondly, tillering is usually inhibited by stem elonga-:tion, which normally follows the transition from the vegetative to the reproductive phase; thus an effect of photoperiod on tillering could be due to a primary effect on floral development. Evans et al. (1964) indicated that generally tillering is increased in short day condi-:tions, although Aspinall and Paleg (1964) suggested that it is the total incident radiation rather than the photo-:period which is significant.

(vi) Temperature

Friend (1965), working on wheat, found that increa-:sing the temperature over the range 10-25°C resulted in an increase in the numbers of both leaves and tillers, although leaf emergence was stimulated to a greater extent than tiller production, and therefore apical dominance was, in effect, increased. Working on ryegrass, Mitchell (1953b) showed a decrease in tillering at higher tempera-:tures, and Cannell (1969b), working on barley, found an overall reduction in tillering at higher temperatures due to differential suppression of the tiller at the coleop-:tile node over that at the first leaf node.

(vii)/

(vii) Hormones

Knowing the major effects of hormones on apical dominance in dicotyledonous plants it might be expected that auxin would have an effect on tillering. However, little critical work on the effects of growth substances on tillering has been done, and evidence of inhibition of tillering by auxin is poor. Leopold (1949) claimed that destruction of the apex of young barley plants increased tillering, while application of the auxin, α-naphthaleneacetic acid, to the destroyed apical region inhibited tillering. It is difficult to see how the apex of a young barley plant could be destroyed without causing extensive damage to the leaf primordia and young leaves enveloping the apex. As the level of tillering was not greater than two per plant, while Leopold claimed he was experimenting on a 'freely tillering species', his work is of doubtful significance, and his paper does not deserve the large amount of attention it has received. Other shortcomings in Leopold's work are that his experiments were carried out using small samples of plants, and were not repeated. Also, he referred to experiments of Cajlachjan and Zdanova (1938) indicating that changes in daylength resulted in changed auxin levels within the plant; then, since he could not show a similar effect in barley, he experimented with Coleus sp., a dicotyledonous plant, and claimed that there was a greater auxin level in the leaves in long day than in short day photoperiodic conditions. He then assumed a similar situation in barley, although/

although he had already failed to find evidence of it experimentally, and suggested that the greater auxin con-:centration in long day conditions was the explanation for increased tillering in short days. Bunting and Drennan (1966) suggest that there is no evidence in Leopold's paper to show that the auxin applied to the destroyed stem apices was not simply toxic, although numerous other workers have quoted Leopold's work without mentioning the inadequacies in it.

Thorne (1962b) and Aspinall (1963) both applied auxin to barley plants at a late stage of development, but no clear effects on tillering were shown in either case. Thorne applied auxin to the cut peduncle of the mainstem, and the coleoptile and first leaf tillers after ear emer-:gence while Aspinall applied it externally.

Recently Jewiss (1972) has described experiments carried out on young wheat plants in which the auxin anta-:gonist tri-iodobenzoic acid (TIBA) was applied to recently initiated tiller buds. It is apparent that the anti-auxin applied to a bud capable of growth caused it to elongate rapidly, although it should be noted that the tiller buds stimulated to develop by this treatment were all capable of elongation without any application of TIBA, so that the treatment affected the rate of tiller elongation, rather than having an absolute effect.

Kirby and Faris (1970) have suggested that at high sowing densities barley plants elongate more rapidly, but tiller only slightly, due to a high internal concentration of/

of gibberellins, although no measurements of gibberellin content were made; other work suggests that the anti-:gibberellin, (2-chloroethyl)-trimethyl-ammonium chloride (CCC) has an inhibitory effect on stem elongation, and a stimulatory effect on tillering (Bokhari and Youngner, 1971 a and b; Humphries, 1968) although Wunsche (1973) found no effect of CCC on two uniculm varieties of barley.

Recently Langer, Prasad and Laude (1973) have given evidence of a stimulatory effect of kinetin on tiller bud extension in wheat, and Ruckenbauer and Kirby (1973) showed that kinetin applied early in the growth of barley increased the number of primordia produced at the apex.

(viii) Other factors.

A number of other factors have been shown to affect the pattern and extent of tillering, including sowing density (Sandfaer, 1953; Kirby and Faris, 1972) and depth of planting (Engledow and Wadham, 1924).

(ix) Summary

The brief survey given above shows clearly that there is a large number of factors affecting tillering; any condition which limits plant vegetative growth adversely affects tillering. Mineral status of the plant, expeci-:ally of nitrogen, phosphorus and potassium, and also the carbon level both have major effects on tillering, and there is increasing evidence for effects of growth sub-:stances. It seems that with reference to the mechanism of tillering the following statement made by Aspinall (1961) is still valid - 'Any scheme which envisages the internal/

internal control of tiller bud elongation by an apical dominance system, and it cannot be denied that such a system does operate within the barley plant ..... must account for the modification of control with changes in the nutrient supply'.

III TILLERING IN RELATION TO CURRENT THEORIES OF APICAL DOMINANCE

Most work published on the control of axillary bud development has referred to experiments on dicotyledonous plants, while the large amount of work on factors affecting tillering has to a great extent been carried out with insufficient consideration to the wider problem of apical dominance. Although there are practical difficulties making it impossible to apply the decapitation techniques used for investigations on dicotyledons to studies on monocotyledons it seems that work on apical dominance and on tillering should be more closely related.

Shein and Jackson (1971) have made a summary, based on a review by Phillips (1969), of the various theories put forward to explain apical dominance as follows:-

1. That removal of the apex makes more nutrients available to lateral shoot growth.
2. That hormones, particularly auxin, attract nutrients to the point of synthesis or application, and that nutrients are diverted away from lateral buds (the nutrient-diversion theory).
3. That auxin directly inhibits the growth of lateral buds.
4. That auxin indirectly inhibits growth of lateral buds by the production of some unidentified compound which inhibits lateral bud growth.

5./

5. That auxin at the apex attracts cyto-:kinins from the root away from the lateral buds, and this lack of cyto-:kinin prevents release of the lateral buds from dormancy.

There is another recent review on the present status of theories of apical dominance by Guern and Usciati (1972).

Shein and Jackson (1971) refer to the need to pay more attention to effects of environment on apical domi-:nance. As a result of their own experiments on Phaseolus vulgaris, investigating effects of soil applied hormones on non-decapitated plants under a range of light intensi-:ties, and with different numbers of leaves removed from the plant, they discard all the above theories and suggest an alternative; namely that it is the balance of various hormones that is important in the control of apical bud development, and that a local change in the balance may allow an inhibited bud to develop.

Recently Tucker and Mansfield (1973) have expressed doubt over nearly all the work so far carried out inves-:tigating apical dominance, since the great majority of experiments has involved either decapitation, or the application of exogenous hormones, or a combination of both procedures. In their experiments Tucker and Mansfield changed the growth pattern of Xanthium strumarium by slightly altering the light regime in which the plants were grown, and then determined hormone concentrations in various organs before and after release of buds from the inhibition due to apical dominance. Tucker and Mansfield/

Mansfield conclude that apical dominance is caused by an indirect effect of auxin, by which abscisic acid is produced in buds causing inhibition, with release from inhibition being possible when the abscisic acid concentration is reduced.

Shein and Jackson (1971) stress the importance of environmental factors in the control of axillary bud development, and recently interest in such a mechanism, involving the nutritional status of the plant and the environmental conditions in which the plant is grown has increased (Gregory and Veale, 1957; Aspinall, 1961; McIntyre, 1964). Species worked on include flax (Gregory and Veale, 1957; McIntyre, 1968), Agropyron repens (McIntyre, 1965, 1969, 1970, 1971a), Euphorbia (McIntyre, 1972), Phaseolus vulgaris (McIntyre, 1973) and pea (McIntyre, 1971b); important effects of nitrogen (Gregory and Veale, 1957; McIntyre, 1965, 1968), and phosphorus (McIntyre, 1968), water stress (McIntyre, 1971b) and various aspects of the light regime (Friend, 1965; McIntyre, 1967; Tucker and Mansfield, 1972, 1973) have all been shown.

Thus it is clear that the phenomenon of apical dominance in both dicotyledons and monocotyledons is considerably affected by nutritional and environmental factors, although the detailed mechanism of axillary bud development is far from being fully understood. It is certain that hormones do have an effect in both types of plant, although only the work of Jewiss (1972) and Langer et al. (1973)/

(1973) is of real significance in relation to tillering, and it seems that mineral nutrition and carbon status could also be of major importance.

IV THE ORIGIN OF THE TILLER BUD AND ITS RELATIONSHIP TO THE MAINSTEM

Sharman (1945), working on Agropyron repens, has shown that the mass of tissue in axillary buds in grasses is produced originally from cells deep within the main-:stem; in contrast leaf primordia are established as a result of divisions of superficial cells in the mainstem.

The precise relationship between a tiller, the main-:stem, and the leaves of the mainstem is far from clear; vascular connections between the various organs are difficult to follow, and the structure of the nodal plexus in grasses is very complex (Majumdar and Saha, 1956; Kumazawa, 1961; Hitch and Sharman, 1971).

Axillary buds are normally initiated 2 - 3 plasto-:chrons later than the leaf in whose axil the tiller is positioned (Bunting and Drennan, 1966); Sharman (1942, 1945) suggests that tiller bud initiation is associated with the encircling growth around the apex by means of marginal meristems of the leaf primordium at the next youngest node; this could indicate that in its origin a tiller is more closely associated with the leaf at the next youngest node than with that in whose axil the tiller is positioned.

Kumazawa (1961), studying Zea mays, and Hitch and Sharman (1968), studying a number of grasses including Dactylis glomerata, Secale cereale, and Lolium perenne have/

have shown that when vascular tissue is first evident in the tiller there is no vascular connection to the mainstem; basi-:petal development of the vascular strand in the tiller bud leads eventually to a vascular link between mainstem and bud. Hitch and Sharman (1971) give evidence that in Poa pratensis the main vascular traces from a tiller anastomose with the nodal plexus at the node of insertion of the tiller, and Inosaka (1958) has shown a similar situation in the rice plant. However the main vascular traces from the leaf do not anastomose with the vascular tissue at the adjacent nodal plexus, and in wheat vascular strands from a leaf eventually fuse completely with other vascular tissue at the nodal plexi up to 3 nodes below the point of insertion of the leaf on the mainstem (Patrick, 1972a). Although some minor vascular bundles become linked to the nodal plexus adjacent to the leaf, fusion of the main bundles from different parts of the leaf occurs at nodes 1-3 below the node of attachment. Patrick's detailed work on wheat made a study of the vascular connections of the older leaves to the mainstem; extensive internodal regions were then present on the stem. Patrick comments that the lower region of the stem consists of very short internodal regions and that the vascular network between lower leaves, tillers and mainstem is 'very complex'. Thus, although the detailed relationship between the various stems and leaves in a grass plant is far from clear, there is evidence from several workers studying a range of grass species that tillers are more closely associated with the leaves at younger nodes on the main-:stem than with the one in whose axil the leaf is posi-:tioned. Bunting and Drennan (1966) state that the mainstem/

mainstem leaf most closely associated by vascular link with a tiller is the one at the next youngest node on the opposite side of the axis, although no reference is given. There is no conclusive evidence of direct vascular connection between a leaf and the tiller developing in its axil.

V RATES OF GROWTH OF TILLERS RELATIVE TO THAT OF THE MAINSTEM, AND DEVELOPMENT OF INDIVIDUAL TILLERS.

Langer (1957) has reported that in Phleum pratense the initial relative growth rate was higher for tillers than for the whole plant, due to the tillers' high initial net assimilation rate. Langer (1959), Ryle (1964) and McIntyre (1965) have all compared rates of appearance of leaves on tillers and mainstem over a range of conditions of nitrogen status. Ryle (1964) reported a 7.3% decrease in the rate of leaf appearance per tiller when nitrogen concentration was decreased from 150 to 15 ppm, while Langer (1959) claimed that the rate of leaf appearance per tiller was unaffected by an increase in the soil nitrogen concentration from 30 to 150 ppm. McIntyre (1965) studied growth of Agropyron repens at concentrations of 210, 10.5, 5.25 and 2.6 ppm nitrogen; he found that initially the rate of leaf appearance showed a positive correlation with nitrogen level, but this did not persist, and eventually the rates were similar at all levels.

In a review of work on the rate of leaf appearance on tillers in grasses Anslow (1966) mentions no instances of differing rates of leaf appearance on tillers in constant/

constant conditions. Both Friend, Helson and Fisher (1962), working on wheat and Cooper and Edwards (1961) working on Lolium found that the rate of appearance of leaves on the mainstem is linear. Mitchell (1953a) recorded a uniform rate of leaf appearance on all tillers, except when the emergence of a tiller was delayed by defoliation; both he and Robson (1974) working on Festuca arundinacea found similar rates of leaf appearance on both mainstem and tillers.

Little work has been carried out comparing rates of development of individual tillers, although a number of workers have compared yields of individual tillers (Cannell, 1969 a and b; Rawson, 1971). In all cases it has been found that the coleoptile tiller gives a smaller yield than the first leaf tiller, although no reasons are given for this effect. Also, the coleoptile tiller has been found to be more adversely affected than the first leaf tiller in poor environmental conditions (Cannell, 1969b), and Jewiss (1972) reported that coleoptile tiller development is less consistent than that for the first leaf tiller.

VI THE IMMEDIATE BACKGROUND TO WORK IN THIS THESIS.

From the previous sections in this introductory chapter, it is clear that a large number of factors are known to affect tillering, and that a great deal of research has been carried out on aspects of tiller growth of economic importance. This has resulted in detailed studies being carried out on the appearance of tillers above their subtending leaf sheaths, but little work has been/

been done at the stage of tiller development between initiation of the bud, and the emergence of the tiller above the leaf sheath. Comparison of the growth of main-:stem and tillers has also received relatively little attention from research workers. A third aspect of tiller growth about which little is known is the effect of hormones on bud development; this lack of informa-:tion is to some extent due to the technical problems of applying hormones to young buds at a developmental stage when they are still surrounded by the mainstem leaf sheaths.

There was therefore adequate scope for a research project on tillering, concentrating on two areas of interest, namely, a study of the growth rate of each tiller compared to that of the mainstem, and the early growth of tiller buds prior to their emergence as tillers above the subtending leaf sheaths. A number of experi-:ments were planned to investigate effects of mineral nutrition on early tiller bud development, and it was hoped that these studies would contribute to the know-:ledge of the mechanism of apical dominance in monocoty-:ledonous plants.

Interest in this department in the growth of barley, Hordeum vulgare, started with Professor H. K. Porter's unpublished experiments on the effects of shading the early formed mainstem leaves. It was already known that for the production of grain in cereal plants, photosynthesis in the flag leaf, the grain itself, and other structures of the inflorescence, is essential (Archbold, 1942; Porter/

Porter, Pal and Martin, 1950; Stoy, 1963; Carr and Wardlaw, 1965; Nosberger and Thorne, 1965; Patrick, 1972b), and that early formed leaves have no direct effect in contributing assimilate to the grain itself. Porter investigated the effects of shading mainstem leaves 1 - 5 on the growth of the whole plant; shading was carried out using shades of aluminium foil, which effectively prevented photosynthesis, but allowed suffi-:cient light to reach the leaf to saturate photomorpho-:genic reactions. She found that by minimising photosyn-:thesis in the first leaf through shade treatment from day 5 after planting, the appearance of older leaves was delayed by up to 6 days, and the grain yield of the plant was significantly reduced. However, shading the first leaf did not reduce the grain yield of the mainstem itself, the reduction in total plant yield being due to the inhi-:bition of tillering. Progressively smaller effects were obtained by shading leaves 2 to 5. Dale, Felippe and Fletcher (1972) confirmed and extended the results of Porter, but found that the delay in appearance of the older mainstem leaves was only about 3 days in plants having their first leaves shaded, rather than 6 days. They showed that reduction in tiller number per plant as a result of shading the first leaf was due mainly to an effect on the secondary and higher order tillers.

Since Porter's investigations more work has been carried out in this department on other factors affecting early growth in barley. Dale (1972) has shown effects of mineral nutrition on photosynthesis in the first leaf and/

and Blenkinsop (1974) has studied effects of shading the first leaf, and of delaying nutrient application on the level and activity in the first leaf of ribulose -1,5 - diphosphate carboxylase, an enzyme important in photosyn- :thesis. Further work on the effects on young plant growth of mineral nutrition, especially of nitrogen (Dale, Felippe and Marriott, 1974), and on development of the mainstem apex (Felippe and Dale, 1973) has also been carried out.

Thus much information has been obtained on factors affecting early development of barley. Tiller bud initiation and early growth are important aspects of plant development occurring during the time that the first leaf is maximally active photosynthetically; it was therefore of interest to investigate tiller growth in plants given treatments involving shading or delayed application of nutrients.

The main aims of the experimental investigation were therefore:-

1. To obtain data on rates of growth of the mainstem and tillers in barley, and to compare various aspects of stem de- :velopment in the different types of stems.
2. To study the early growth of tillers prior to their emergence above their subtending leaf sheaths, with special reference to the effects of shading the first or second mainstem leaf, and of delaying or withholding mineral nutrient application, on young tiller bud growth.

One set of results reported in Dale, Felippe and Fletcher (1972), and all the data presented in Fletcher and/

and Dale (1974) were obtained during the course of this project. All the results in the latter paper are also fully discussed in this thesis; the sets of data (Results, Section 3, Tables 2 and 3) in Dale *et al.* (1972) referring to the effects of shading either the first or second leaf on tillering in barley are again presented here, although other results in the paper are referred to in the text of this thesis without full discussion. Copies of both papers are included at the end of this thesis (Appendix B).

CHAPTER 2

MATERIAL AND METHODS

I THE MATERIAL

Barley, Hordeum vulgare L.,of the cultivar Proctor, was used throughout the experimental investigation. It was felt that variation in grain and plant growth characteristics would be greater if different batches of grain were used from harvests each year, than if grain from a single harvest were used as far as possible, even though the viability of grain from a single batch might be reduced over the 4 years of the project. Therefore, for most of the experiments grain supplied by the Scottish Plant Breeding Institute, Pentlandfield, harvested in 1970 was used; however, for the initial experiment, investigating the effects of shading either the first or second leaf on the rates of appearance of leaves on mainstem and tillers, grain of the 1969 harvest was used, since Dale and Felippe (Dale, Felippe and Fletcher, 1972) were using that batch of grain for their work at that time. Towards the end of the project the supply of grain from the 1970 harvest was nearly exhausted, and therefore grain supplied by the Plant Breeding Institute, Cambridge, harvested in 1970, was used for the experiments investigating the uptake of $^{14}CO_2$, and its transport to the tillers. All the batches of grain were stored in a refrigerator at a temperature of approximately 8 - 10°C.

II THE METHODS

(i) Conditions of plant culture

(a) The choice of medium

As/

As a large part of the experimental investigation was concerned with studying the effects of nutrient appli-:cation on growth of the plant, it was necessary to culture the plants in a medium essentially nutrient-free; this necessity eliminated both compost and soil as possibili-:ties. Vermiculite is known to have a variable mineral composition, and growth of plants for more than about 10 days in peralite (British Gypsum Ltd., Wetherall, Carlisle) was unsatisfactory so neither of these media was used to any extent, although peralite was used for one experiment. Washed river sand, Quartzag B supplied by Quartzag Ltd., Blanefield, was found to be the most suitable growing medium; for some batches of sand addi-:tional washing was necessary to ensure uniform and healthy plant growth.

(b) The choice of container

Throughout the investigation plastic pots having a volume of about 250ml were used. Initially, round pots with a diameter of 9cm were used, but when supplies of these became short a stock of square pots, of the same volume, and with a side length of 9cm was used. Except for one experiment at the start of the project, when two grains were planted in each pot and then thinned to one seedling per pot on day 4, a single grain was planted in each pot at a depth of 2.5 - 3 cm. It was found that planting one grain per pot produced more uniform plant growth than using a larger container having several seedlings, although it limited the number of plants that could be grown.

(c)/

(c) Selection of grain and seedlings

Selection of plants for experimental work was carried out at three stages. Firstly, all grains showing evidence of bacterial contamination, physical damage, or other imperfection were rejected. Secondly, only those grains in the weight range 45 - 55 mg were used; and Thirdly, on day 4 after planting, pots in which there was no visible seedling, and those in which seedlings were abnormally large or small were rejected. Selection at the first and third stages was carried out throughout the project, and rejection of grains outside the weight range was used in most, but not all, of the experiments. Using these pro-:cedures it was possible to obtain about 150 suitable plants out of a total of 187 pots that could be positioned on the growth room table. With the PBI, Cambridge grain, harvested in 1969, the proportion of suitable plants was slightly higher. Even with these fairly stringent pro-:cedures for selection, it was impossible to obtain as uniform a population as would have been wished, and there was still a considerable amount of variation in the material, particularly in the later stages of the experi-:ments. In most of the experiments it was not possible to grow spare plants, due partly to the limited space available, and also because of the number of treatments being given. Thus, any plants showing signs of abnormal growth only after day 4 had to be excluded from the experiment and, generally, were not replaced.

(d) Water and nutrient application

At planting, and also normally on day 2 plants were given/

given 50ml distilled or deionised water. For the stan-:dard treatments 50ml of a modified Hoagland's nutrient solution were applied on day 4, as described by Dale et al. (1972), and similar also to the regime used by Porter in her experiments. Amounts of each mineral in the nut-:rient solution are shown in Table 2.1; the full nutrient solution was applied on days 4, 11 and 18, and non-nitro-:genous nutrients only on days 25 and 32. Non-nitrogenous nutrients with half the standard amount of nitrate, and half supplies of nitrate were applied on days 39 and 46 respectively, and thereafter no nutrients were applied. Throughout the growth of the plant water was added when necessary; usually 50ml every second day, and more frequently as the plants grew older. With the above nutrient regime the standard application per plant con-:tained amounts of major elements as follows:- N, 14.0mg; P, 3.2mg; K, 43.0mg; Mg, 2.9mg; Ca, 10.0mg. Iron, as the versenate salt, and micronutrients, as in Hoagland and Arnon (1938) were also supplied. For experiments in which variations on the standard nutrient application were used, details are given as the results of the experi-:ments are discussed in the relevant sections later in this thesis.

(e) Growth room conditions

All the plants used in experiments were grown in constant environment rooms, having a central table whose height could be raised or lowered to vary the light intensity. There was sufficient space within the growth room to walk round the table, so that plants could be inspected/

Table 2.1 Components of the standard mineral nutrient solution supplied to plants. Micronutrients, as in Hoagland and Arnon (1938) were also supplied with the non-nitrogenous minerals.

	Stock Solution	Strength of stock Solution	Volume (ml) of stock solution per litre nutrient solution
Non-nitrogenous minerals	K_2SO_4	0.5 M	20
	KH_2PO_4	0.2 M	10
	$CaCl_2$	0.5 M	10
	$MgSO_4$	0.4 M	10
	EDTA	0.025%	10
Nitrogen	$NaNO_3$	1.0 M	20

inspected and manipulated as necessary. The light intensity, measured by an EEL lightmaster photometer calibrated against a Lintronic thermopile, was 1800-3500fc in the wavelength range 400-700nm, and was produced from warm white fluorescent tubes and tungsten filament lamps. This intensity was equivalent to about 63-122 Wm^{-2}, or 86-168 cals cm^{-2} $16h^{-1}$. Plants were illuminated for a 16h day, and the temperature was regulated to $20\pm1^{o}C$ day and night, except that in the experiment investigating rates of leaf appearance on the mainstem and tillers, one set of plants was grown in a temperature regime of $20^{o}C$ during the day and $17^{o}C$ at night.

(f) Randomisation of experiments

Even in the controlled environment rooms there were gradients over the table of various conditions; light intensity decreased from the centre of the table to the extremities, and there was a gradient across the table in the speed at which the pots dried out, due to the air circulation within the room. It was essential therefore that the positions of the plants on the table were randomised. This was done as far as possible, although the need for randomisation had to be balanced against the need to apply different treatments to the plants, and the consequent possibility of making errors in application in fully randomised arrangements.

(g) Shading technique

Tubular shades of aluminium foil were applied to the first or second leaf as described in Dale et al. (1972), and were generally 20cm in length, as used by Blenkinsop/

Blenkinsop (1974). The shade was applied to the first leaf on day 5 after planting, and its height was kept level with the ligule by sliding the shade up the stake to which it was attached; the second leaf was allowed to develop outside the shade after its appearance above the ligule of the first leaf on about day 8-9. Where the second leaf was shaded this was done from appearance on day 7-8 and throughout development, the third leaf being allowed to develop outside the shade after its appearance on day 13-14. Light intensities within a 14cm shade are given by Dale et al. (1972) and show very low intensities (less than 100fc) within 4cm of the open end. The expan-:ding region of the leaf, at the basal meristem, there-:fore always developed in conditions of extremely low light intensity, and the shaded leaf was capable of neg-:ligible photosynthesis, although it is thought that sufficient light would reach the leaf to saturate low intensity photomorphogenic reactions (Dale et al, 1972).

(h) Sample size and analysis of results

For most of the experiments samples of 8-10 plants were used at each time of harvest for each treatment, although the exact number depended on the particular experiment, and is given when results of each experiment are discussed. Occasionally the sample size had to be reduced because of an abnormal plant, but this was not enough to affect seriously the interpretation of results. Over the whole project it was found that 80 out of a total of 3206, or 2.5%, of the plants dissected had two tillers at the coleoptile node, although no plants were ever/

ever found with two tillers at any other node; where two coleoptile tillers were present in a single plant the results were excluded from the sample.

Where dry weights of tillers and plants were obtained as a measure of growth in particular conditions, the data were transformed to their respective $\log_{10}$ values, as an essential preliminary to statistical analysis and other processing of the data, before mean dry weights of samples were calculated. This procedure made the sample values more nearly 'normal' in their distribution.

To aid in the interpretation of results standard statistical procedures have been used as appropriate. In experiments measuring dry weights of structures the statistical procedures were carried out on the $\log_{10}$ data. During the project the following methods have been used:-

1. Coefficient of Variation. The ratio of standard deviation to mean, expressed as a percentage.
2. 95% Confidence Limits. These were calculated as the mean ± the product of the standard error and $t(p = 0.05)$ for appropriate degrees of freedom. A difference between two samples was taken to be significant if the calculated 95% confidence limits of each sample did not include the mean value of the other sample.
3. Analysis of Variance. When it was necessary to compare results from more than two samples, analysis of variance methods were used, and least significant differences calculated to indicate significant differences between samples.
4. Linear Regression Analysis. To compare rates of appearance of leaves on various stems, and rates of growth in structures increasing in dry weight exponentially, equations for the linear regressions were calculated. Comparison of/

of the slopes of these regressions was possible using the analysis of variance technique.

(ii) Nomenclature and labelling of plant parts

Tiller nomenclature is similar to that used by Kirby and Faris (1972), Jewiss (1972) and Rawson (1971), in which the tiller carried in the axil of the coleoptile is designated TC, and the first, second and third leaf tillers are designated T1, T2 and T3 respectively. A similar notation is used for the secondary and higher order tillers, so that T1.P is the secondary tiller pro-:duced in the axil of the prophyll of T1. The nomencla-:ture used is somewhat clearer than that used by Thorne (1962 b), Cannell (1969 a and b) and Dale et al. (1972).

In the experiment following rates of appearance of leaves on mainstem and tillers on the plant to maturity the fourth, sixth and eighth leaves on each stem were marked with 1, 2 or 3 lines respectively at their tips using Indian ink; tillers were labelled using a colour code to signify their position, by means of coloured plastic rings cut from plastic straws and put over the tiller on the day of its appearance above its subtending leaf sheath. Dates of appearance of leaves and tillers were determined by daily or bi-daily observations.

(iii) Procedures followed to obtain dry weight data

(a) The dissections

1. Of young tiller buds

The dissection under a Vickers sterimag microscope involved careful removal of the coleoptile and sheaths of the first and second leaves by means of a fine needle and/

and watchmakers forceps to expose tiller buds TC, T1 and T2 respectively. By means of a tool made from a surgical needle mounted in a holder and filed to give a fine cutting edge the tiller buds were cut from the stem.

Leaves on the barley plant are initiated in two ranks in a single plane alternately on either side of the stem. Tiller buds are initiated in the same plane as the leaves; the position of each tiller bud is just above the node at which its subtending leaf is attached, on the side of the stem from which the leaf was originally initiated, and on which the lamina is displayed. In the case of TC, the bud is positioned in the axil of the coleoptile, underneath the scutellum.

2. Of whole plants

Roots were washed free of sand, and the plants divided into their constituent parts, the leaves, bases, and roots. The bases included all the leaf sheaths, the parts of the plant within the youngest visible leaf sheath, and also the remains of the grain husk. In the experi-:ments in which all the leaves were dissected from the stem individually, the dissecting instruments described above were used for the youngest leaves.

(b) Methods for obtaining dry weights

All the material used to obtain dry weights was heated in an oven at 90°C overnight. The tiller buds were placed on a watchglass or in small vials, and the large plant parts were positioned on baking trays. Young tiller buds and leaves having a dry weight of less than 1mg were weighed on a Sauter torsion balance to the nearest/

nearest 2μg. It was impossible to weigh accurately below about 10μg, so that the very smallest buds and leaves had to be weighed in groups. Material weighing over 1mg was weighed on an Oertling electric balance, accurate to within 0.1mg.

(iv) Procedures used in observations of various aspects of tiller development

(a) Methods used in sectioning and staining

Material was dissected from the plant using the same instruments as described above, and fixed, embedded, sectioned and stained using a procedure modified from Johansen (1940), as outlined in Fig. 2.1. A staining procedure prior to sectioning was used to facilitate orientation of the tissue within the paraffin wax block. In a second batch of sections the counter staining in light green was not carried out, as it was found to be unnecessary for the purposes of this investigation.

Sections were cut at a thickness of 10μm using a Beck rotary microtome, and were then examined to observe numbers of leaf and tiller primordia present. Camera lucida drawings were obtained for sections showing features of particular interest, and these are shown in the relevant section of the thesis.

(b) Estimation of apical dome size

Mainstem and tiller apices were exposed under the dissecting microscope using fine instruments, and were then viewed under a microscope. Camera lucida tracings were made of the outline of the apical region, and from these tracings the length and breadth of the apical dome were/

Figure 2.1 Procedure used for fixing, embedding and staining material for sectioning.

Fixation	3:1 ethanol : acetic acid	1 h
Prestaining	90% ethanol	10 mins
	70% "	"
	safranin	"
Dehydration	70% ethanol	10 mins
	90% "	"
	100% "	"
	100% "	"
Embedding	2:1 ethanol : chloroform	½ h
	1:1 " : "	"
	1:2 " : "	"
	100% chloroform	"
	100% "	"
	Infiltration with wax shavings	overnight
	3 changes of wax	
	material placed in wax block	
Sectioning	material sectioned at thickness 10 μm	
Staining	100% chloroform	10 mins
	100% ethanol	"
	70% "	"
	safranin	"
Mounting	70% ethanol	
	90% "	
	100% "	
	100% "	
	Counterstain in light green	c.1 min.
	2 changes in clove oil	
	mounted in balsam	

were found; the apical dome is defined as the region above the youngest leaf primordium (Fig. 2.2). The area of profile of the apical dome was found by measuring the weight of paper representing the apical dome, and converting this value by means of the known weight of paper per unit area.

(c) Comparison of the rate of primordial initiation on mainstem and tillers

Mainstem and tiller buds were dissected carefully so that numbers of leaves, and of leaf and floral primordia could be ascertained; in this investigation one spikelet was regarded as a single floral primordium. It was difficult to recognise the floral primordia under the dissecting microscope, until they were clearly developed to the 'double ridge' stage, and therefore the results are shown as 'total primordia number' at each time of harvest. Further details of the methods used to express the data are given when the results are presented (Chapter 3, page 76).

(d) Estimation of the stage of apical development

After careful dissection using fine instruments, mainstem and tiller apices were observed under a microscope. The stage of development of the most advanced primordium was classified according to the scheme of Aspinall and Paleg (1963) (see also Table 3.8, page 71).

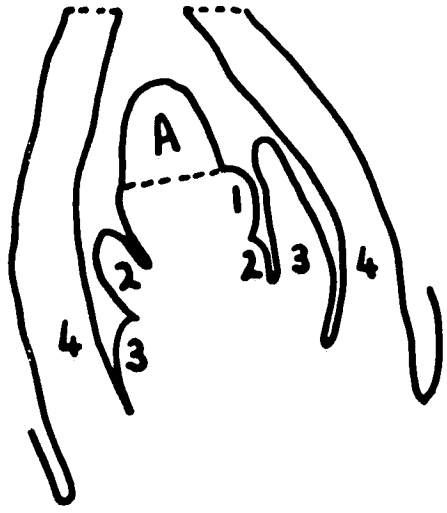
(v) Method of application of $^{14}CO_2$ to the barley plant

A considerable amount of time was spent in developing a suitable method for application of $^{14}CO_2$ to young barley plants, so that transport of assimilate from/

Figure 2.2 Diagrammatic median longitudinal section of mainstem apical region of young barley plant.

A = Apical dome; 1-4 = leaf primordia 1-4.

Fig 2.2



from particular leaves into the tiller buds could be followed.

The first method of application attempted used apparatus as described in Felippe and Dale (1972); a perspex box, volume 12.3 l, was connected to a circulatory pump, and to a small vessel in which $^{14}CO_2$ (150μCi) was liberated from NaH $^{14}CO_3$ (specific activity 57mCi $mmol^{-1}$) by addition of lactic acid. Four plants were placed in the airtight box and then exposed to $^{14}CO_2$ by circulating the gas in the closed system for 10 minutes; the circuit was then opened and laboratory air passed through the box, and through a potassium hydroxide trap. This purging lasted 50 minutes, and throughout it and the presentation period the plants were irradiated at saturating light intensities of 240 W m^{-2} from a 'Zeiss Ikomat' projector. The sides of the box were covered with highly reflective 'Melinex' to maintain the maximum possible light intensity within. After the initial purging, the box was removed from the fume chamber, in which all the preceding operations had been carried out, and placed in the growth room in which the plants had originally been grown, until the time of harvest. The main disadvantage of this method was that four plants were exposed to the $^{14}CO_2$ together, and it was uncertain if plants had received identical amounts of $^{14}CO_2$; it was also difficult to apply exactly similar quantities of the label to each set of four plants in different boxes. Very variable results were obtained for the amounts of labelled assimilates being transported to individual tillers, and in nearly a third of/

of the cases the confidence limits exceeded the mean value (Table 2.2). This method was therefore abandoned, and one developed in which plants could be individually supplied with $^{14}CO_2$.

This second method involved exposing plants to $^{14}CO_2$ displaced from a large, closed, glass flask, and passing it into a chamber covering the plant, which was illuminated by a projector, as in the method previously described. A diagram of the apparatus is shown in Fig. 2.3.

One mCi of $NaH^{14}CO_3$ (specific activity 57 mCi $mmol^{-1}$) was dispensed into a 10 litre round bottomed flask, and the neck sealed by means of a rubber bung. Two glass tubes, each with a tap, passed through the bung; the first, from a reservoir allowed addition of liquid into the flask, and the second was an outlet tube to allow displaced gas to be passed from the flask. The plant was covered by a cylinder, of internal diameter 14mm, and made of perspex 3mm thick. One side of the cylinder was covered by a reflecting surface of 'Melinex', to allow maximum illumination of the leaf by a projector positioned 40cm away, and producing a light intensity of approximately 200 W m^{-2} at the leaf surface. The cylin-:der covering the plant had its bottom end pushed about 1cm into the sand, and was sufficiently long to allow the plant to be positioned without damage; it had two points at which gas could be passed in or out. The first of these, for gas input, was connected by rubber tubing to the outlet tube from the 10 litre flask, and the second/

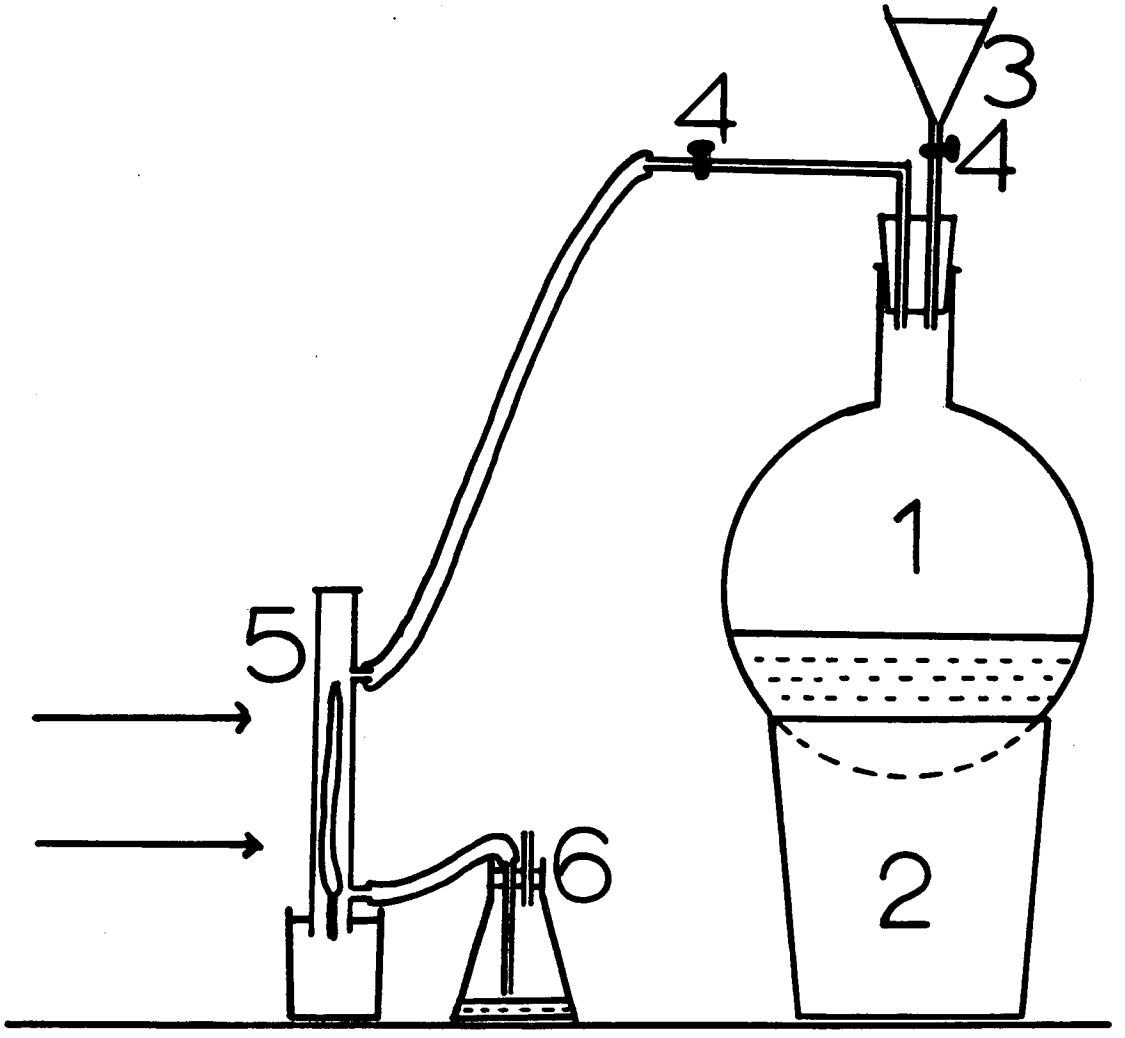
Table 2.2 Radioactive counts per tiller in TC and T1 up to 24h after application of $^{14}CO_2$ to plants 6, 8, 10 or 12 days old; 95% confidence limits are indicated.

Age of plant (days)	Time between ^{14}C application and harvest (h)	TC	T1
6	1	116 ± 292	112 ± 95
	3	60 ± 92	190 ± 266
	6	92 ± 106	52 ± 58
	24	62 ± 96	291 ± 406
8	1	285 ± 258	379 ± 236
	3	1559 ± 897	1057 ± 826
	6	1020 ± 1154	518 ± 510
	24	184 ± 106	195 ± 163
10	1	446 ± 269	1014 ± 375
	3	1003 ± 524	1603 ± 476
	6	529 ± 156	282 ± 210
	24	978 ± 194	1847 ± 561
12	1	36 ± 59	217 ± 124
	3	696 ± 414	654 ± 631
	6	707 ± 701	761 ± 387
	24	1584 ± 2801	907 ± 268

Figure 2.3 Apparatus used for the application of $^{14}CO_2$ to young barley plants.

1 = 10 litre flask; 2 = bucket, to support flask;
3 = funnel; 4 = tap;
5 = perspex cylinder covering plant; 6 = KOH flask.

Fig 2.3



second, towards the bottom end of the cylinder, allowed gas to escape after passing over the plant.

The labelled carbon dioxide was liberated from the $NaH^{14}CO_3$ by addition of a small amount of 1% lactic acid, and then each plant was exposed to 100 ml gas containing $^{14}CO_2$, by addition of 100 ml of lactic acid from the reservoir, over a period of two minutes. Application of $^{14}CO_2$ to plants was carried out inside a fume chamber, and plants were then returned to the growth room until the time of harvest. Unpublished data have shown that up to 50% of the carbon fixed by the plant is liberated over 24h following application, and therefore to prevent escape of $^{14}CO_2$ into the growth room atmosphere each plant was placed in a polythene bag. However, disadvantages were found with this method; firstly, using polythene bags meant that plants were not given a satisfactory supply of air during the period between application of the $^{14}CO_2$, and harvest. A second disadvantage was that since the application of $^{14}CO_2$ was carried out inside a fume chamber in a laboratory away from the growth room, plants were subjected to a change in environmental conditions on their removal from the growth room. A third disadvantage was found when it was discovered that the amount of radioactive label in each sample of 100 ml gas from the flask diminished as the flask was filled up with lactic acid; it was found that a proportion of the counts were going into the lactic acid, due probably to either bacte-:rial or fungal contamination.

To overcome these inaccuracies the method was adapted/

adapted as follows:- Firstly, to ensure a sufficient supply of air to the plants over the period between application of $^{14}CO_2$ and harvest, plants were placed in the perspex boxes, as described by Felippe and Dale (1972), and air passed through the boxes. Secondly, to ensure minimal environmental changes for the plant, application of the $^{14}CO_2$ was carried out in the growth room; extra safety precautions were taken involving a potassium hydroxide trap after gas had passed over the plant, to prevent $^{14}CO_2$ reaching the atmosphere (Fig. 2.3). The third disadvantage of the method described above was overcome by using a dilute (0.01N) solution of hydrochloric acid instead of 1% lactic acid to displace samples of gas from the flask. The volume of gas liberated for each plant was increased to 150ml, and the exposure time was $1\frac{1}{2}$ minutes.

Studies were carried out on plants up to day 14, at which time the third leaf was just appearing on the mainstem. To investigate transport of labelled assimilate to the tillers from either the first or second leaf, only one or other leaf was allowed to take up $^{14}CO_2$; photosynthesis in the other leaf was prevented by covering it with a cylindrical shade made of aluminium foil, and having a diameter very slightly greater than the width of the leaf. The third leaf was also shaded. Care was taken to ensure that as far as possible the shade covering one of the leaves did not reduce the light intensity on the unshaded leaf.

At harvest plants were dissected, and each tiller removed and placed in a scintillator vial containing 1.0ml

2N potassium hydroxide; the vial was then placed in an incubator at 37°C overnight. Nine millilitres of scintil-:lator liquid were then added to each vial; this liquid was made up of a mixture of toluene and triton X-100 (Intertechnique Ltd.) in the ratio 2:1 parts by volume, and containing 2(4-tert-butylphenyl)5(4-biphenylyl) 1, 3, 4 oxadiazole (butyl PBD) at a concentration of 8gpl. The solubilisation procedure, and scintillation liquid mix-:ture were recommended by Dr. J. Ingle, and a similar procedure has been described by Gore (1973). The scintil-:lation liquid mixture was shaken thoroughly to obtain a clear solution, and cooled before counting for 10 mins, on a Packard Tri-carb scintillation counter.

CHAPTER 3

ASPECTS OF GROWTH OF MAINSTEM AND TILLERS IN BARLEY

There have been few detailed comparisons of rates of growth of the mainstem, primary and secondary tillers on grass plants. Mitchell (1953a) and Robson (1974) found no differences in rates of appearance of leaves on main-:stem and tillers in *Lolium perenne* and *Festuca arundina-:cea* respectively; Langer (1957) working on *Phleum pratense* found that tillers had a higher initial relative growth rate than the whole plant. As far as is known, no work has been carried out comparing aspects of apical development on mainstem and tillers.

Dale *et al*. (1972) showed that shading the first leaf of Proctor barley reduced plant grain yield, due to a reduction in the number of tillers developing to matu-:rity; treatment was also found to delay the appearance of tillers above the sheaths of their subtending leaves. Shading the second leaf had much smaller effects on tiller development and final grain yield.

The main objectives of the work to be described in this chapter were therefore to compare rates of growth of mainstem and tillers in barley, in terms firstly of rates of appearance of leaves, and secondly, of relative growth rates, and also to make a comparative study of apical development. Additionally, further data on the effects of shading the first or second leaf of barley on the initiation, dates of appearance, and yields of tillers at specific positions on the mainstem were obtained,/

obtained, and during the course of the work some effects of grain ageing were studied.

I GROWTH TO MATURITY OF MAINSTEM AND TILLERS IN BARLEY, AND THE EFFECTS OF SHADING THE FIRST OR SECOND LEAF ON THIS

Dates of appearance of tillers, and of leaves and awns on all stems were found by making observations at least every two days on samples of 10 plants for each of the three treatments, involving shading the first or second leaf, and untreated controls; final grain yields per stem were also measured. All the results to be discussed in detail were from plants grown in a controlled environment room with a 16h photoperiod, and temperature of 20°C day and night. Some previous work (Dale et al., 1972) involved growing plants in a temperature regime of 20°C during the day, and 17°C at night, with other conditions as described above; therefore sets of plants of the three treatments were grown in both temperature regimes to check that there were no gross differences in growth. The results from the plants grown in the 20/17°C regime are shown in Appendix A, and a brief comparison with the 20/20°C regime results is made in the text of this chapter (Section I (vi) page 65).

(i) The appearance of leaves on the mainstem

The date of appearance of the second leaf did not differ in the three treatments (Table 3.1). Appearance of the third leaf was delayed 2.8 days when the first leaf was shaded, but was not affected by shading the second leaf. The two treatments delayed the appearance of the fourth leaf by 4.0 and 1.2 days respectively, and similar/

Table 3.1 Age of plant (days) at the time of appearance of leaves 2 - 10 on the mainstem in control plants, and those having either the first or second leaf shaded.

Mainstem leaf number	Control	Shaded	
		First leaf	Second leaf
2	7.7	8.3	8.0
3	13.6	16.4	13.3
4	19.0	23.0	20.2
5	24.1	27.8	25.4
6	29.2	33.0	31.2
7	35.6	38.7	36.7
8	40.8	44.7	41.9
9	46.7	50.7	48.0
10	52.4	55.3	53.6

similar delays persisted throughout the growth of the plant so that appearance of the tenth leaf was delayed by 2.9 and 1.2 days where the first or second leaves were shaded. Apart from the initial perturbation caused by shading either the first or second leaf the appearance of leaves on the mainstem was regular, with an interval between successive leaves of 5 - 6 days.

Thus a persistent, long-term effect, resulting in delayed leaf appearance throughout plant growth, was caused by shading either the first or second leaf, although the former treatment caused a more severe check than the latter.

(ii) The appearance of tillers

Only those tillers appearing above their subtending leaf sheaths in at least 50% of the plants of a sample contribute to the data in Table 3.2. In control plants the coleoptile and first leaf tillers, TC and T1, became visible within approximately a day of each other at about day 20 after planting, and primary tillers appeared at the higher nodes in acropetal succession. Tillers T2, T3 and T4 emerged 5.6, 9.9 and 19.7 days after T1 in the control treatment, giving no evidence of a regular time interval between the emergence of successive tillers comparable to that for leaf appearance. Delay in the emergence of T1 due to shading the first or second leaf was 6.5 or 5.6 days respectively, and corresponding values for T2 were 3.4 and 1.9 days. Shading the first leaf had less marked effects on delay in tiller appearance at the higher nodes, and shading the second leaf caused no delay in/

Table 3.2 Age of plant (days) at the time of appea-:rance of tillers, in control plants, and those having either the first or the second leaf shaded.

Tiller	Control	Shaded	
		First leaf	Second leaf
TC	21.4	—	—
T1	20.3	26.8	25.9
T2	25.9	29.3	27.8
T3	30.2	33.3	33.0
T4	40.0	44.0*	38.5
T1P	33.6	39.7	32.6
T2P	36.0	37.0	37.1

*Appeared in only 4 plants

in T4 emergence. In the case of secondary tillers, emergence of that in the prophyll of the first leaf tiller, T1.P, was delayed to a greater extent than that of T2.P by shading the first leaf, with delays of 6.1 and 1.0 days respectively being observed. This considerable delay in T1.P emergence resulted in its becoming visible after T2.P, so reversing the order of emergence found in control plants. Shading the second leaf had no effect in delay-:ing the appearance of T1.P, and only a slight effect on T2.P.

In control plants, the order of appearance of tillers was TC/T1, followed by T2, T3, T1.P, T2.P and T4; this order is very similar to those found in cv Spratt Archer and Maris Badger (Cannell, 1969a) and in Plumage Archer and Proctor (Thorne, 1962b).

The cultural conditions, especially of mineral nutri-:tion, restricted tillering (cf. Aspinall, 1961), and in the control treatment an average of 7.1 visible tillers were produced on each plant (Table 3.3). Shading had an effect on tillering, reducing numbers of visible tillers produced by about 25% and 10% where first or second leaves were shaded. There were no reductions in numbers of T1, T2 and T3 tillers due to shading either the first or second leaf; however, shading the second leaf reduced the number of TC tillers appearing, and shading the first leaf led to a smaller number of TC, and, curiously, T4 tillers. The data also show that shading the first leaf reduced numbers of secondary and higher order tillers appearing, although this result did not reach a significant level./

Table 3.3 The numbers of tillers at particular positions appearing per plant in control plants, and those having either the first or second leaf shaded. Numbers in parenthesis indicate the numbers of tillers per plant surviving to maturity; 95% confidence limits are indicated.

Tiller	Control	Shaded	
		First leaf	Second leaf
Primary tillers			
TC	0.8	0.2	0.3
T1	0.9	0.9	0.9
T2	1.0	1.0	1.0
T3	1.0	0.9	0.9
T4	0.9	0.4	0.9
Total	4.6 ± 0.4 (4.1)	3.4 ± 0.4 (3.1)	4.0 ± 0.4 (3.5)
Secondary & higher order tillers from			
TC	0.3	0.3	0.3
T1	1.2	0.8	1.0
T2	0.8	0.8	1.2
T3	0.2	0.1	0.3
Total	2.5 ± 1.1 (0.6)	2.0 ± 0.9 (0.6)	2.8 ± 0.7 (0.6)
Total tillers per plant	7.1 ± 1.3 (4.7)	5.4 ± 1.1 (3.7)	6.8 ± 0.8 (4.1)

level. The proportions of the total visible tillers surviving to maturity in control, and treated plants having their first or second leaves shaded were 0.66, 0.68 and 0.60 respectively; there is therefore no evidence of an effect of shade on the proportion surviving.

Thus, shading the first leaf of barley caused delays in dates of appearance, but did not alter the order of emergence of the primary tillers, whereas emergence of the secondary tiller T1.P was delayed to such an extent that it appeared after T2.P. Shading the second leaf caused smaller delays in the dates of tiller appearance, but the order of appearance was the same as that in control plants. Shading the first, or to a lesser extent the second leaf, reduced the numbers of tillers appearing, but neither treatment affected the proportion surviving to maturity.

(iii) Rates of leaf appearance on mainstem and tillers.

Rates of leaf appearance on the mainstem and successive primary tillers for the control and shaded treatments are shown in Fig. 3.1. The correlation coefficients for the linear regressions of the rates of leaf appearance on both mainstem and tillers were all above 0.99, indicating that the fitted straight lines account for a very high proportion (over 98%) of the variation in the data.

The linear regressions (Fig. 3.1; Table 3.4) were calculated using the appearance dates of all leaves on the mainstem except the first, in the treatment involving shading the second leaf and the untreated controls.

Values/

Figure 3.1 Rates of leaf appearance on the mainstem and tillers in control plants (A) and those having either the first (B) or second (C) leaf shaded.

Fig 3.1

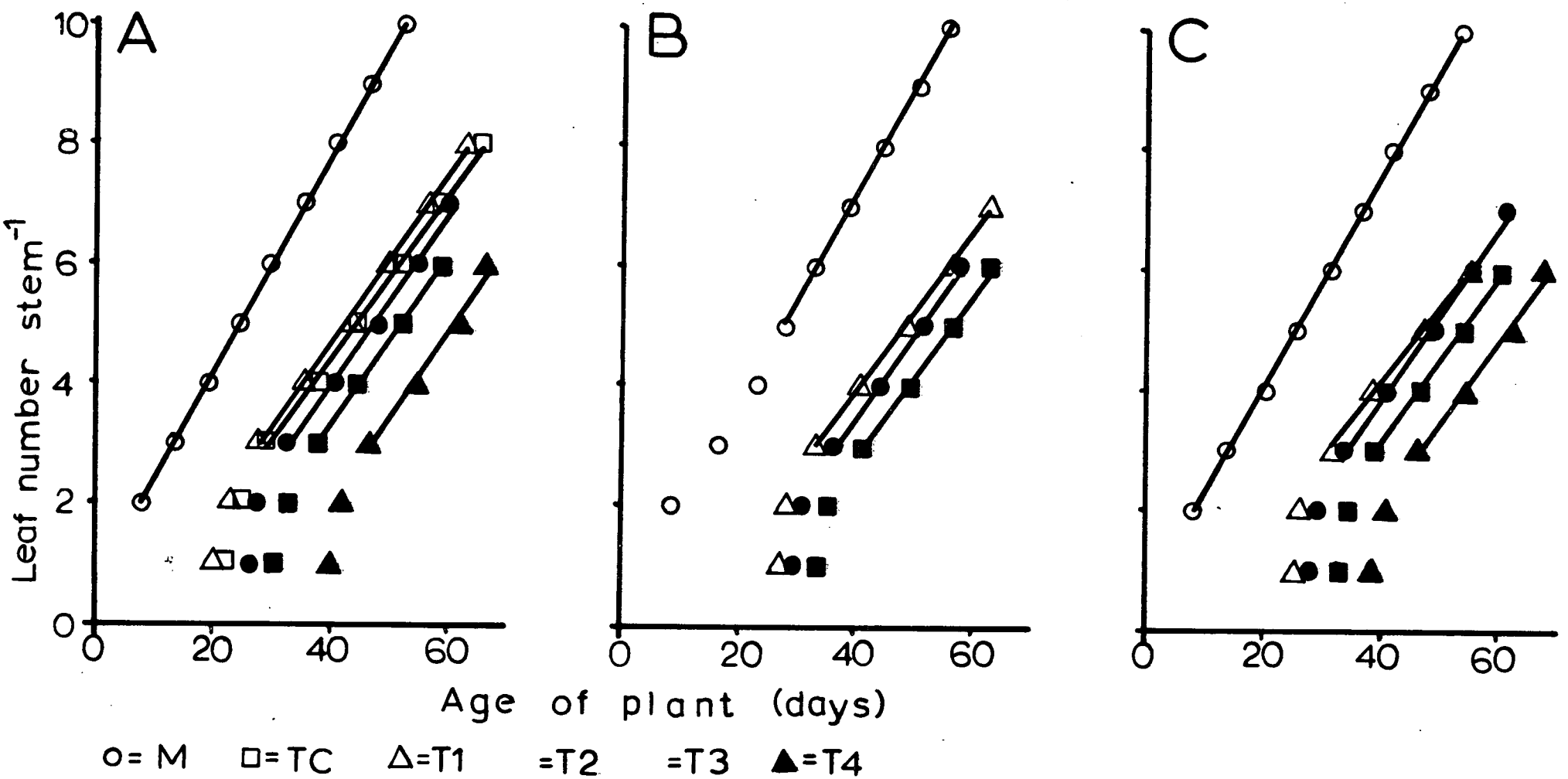

A
B
C
Leaf number stem^{-1}
0
2
4
6
8
10
0
20
40
60
Age of plant (days)
o= M
□= TC
Δ=T1
=T2
=T3
▲=T4

Table 3.4 Regression coefficients for the rates of leaf appearance on the mainstem and tillers in control plants, and those having either the first or second leaf shaded. Correlation coefficients for all the regressions were greater than 0.99.

Stem	Control	Shaded	
		First leaf	Second leaf
M	0.180	0.178	0.176
TC	0.137	-	-
T1	0.142	0.132	0.127
T2	0.143	0.140	0.144
T3	0.141	0.136	0.138
T4	0.148	-	0.137
T1.P	0.121*	0.121	0.111*
T2.P	0.123	0.148	0.121*

* indicates a significantly lower value ($p = 0.05$) of the regression coefficient of a secondary tiller compared with that of the parent primary tiller.

Values for leaves 2, 3 and 4 were omitted from the calculation in the treatment involving shading the first leaf, since the check in growth referred to above (page 48) resulted in a marked divergence from linearity over these points. Since the first and second leaves on the tillers were well developed, and appeared very soon after emergence of the tiller, the data on the appearance of these leaves were omitted from the calculations of the linear regressions. The regressions of the rates of leaf appearance on the primary tillers were calculated for only those tillers which appeared and grew to maturity in at least 50% of the plants of a particular treatment. In the case of secondary tillers, the data available were much less extensive, due to the large proportion that senesced before earing, and therefore the dates of leaf appearance from as few as 3 or even 2 secondary tillers at a particular position were used to calculate the linear regressions of the rates of leaf appearance. Thus, the results obtained for the secondary tillers are unlikely to be as accurate as those for the mainstem and primary tillers.

From Table 3.4 it is seen that the values for the slopes of the linear regressions for leaf appearance on the mainstems were similar for control and shaded plants, averaging 0.178. This value for the mainstem was higher than those for the primary tillers, which ranged from 0.127 to 0.148, averaging 0.139. Comparisons of the slopes for mainstem with those for their primary tillers all showed a significantly higher rate of leaf appearance on/

on the mainstems ($p = 0.001$), but there were no significant differences between tillers T1, T2 and T3 within a single treatment. Results for TC were scarce, due to the large number that senesced before earing, but the values obtained indicate that the rate of leaf appearance on TC tillers growing to maturity was similar to that on the other primary tillers. The values of the slope measuring rate of leaf appearance on T4 was also similar to those of the other primary tillers. No significant differences were found in comparisons of rates of leaf appearance on T1.P and T2.P within the same treatment, and the range of slopes for the secondary tillers was 0.111 - 0.123, apart from one anomalous value. There was a significantly lower rate of leaf appearance ($p = 0.05$) on a secondary tiller compared to its parent primary tiller in 3 out of 6 comparisons.

From the values of the slopes of the linear regressions it can be calculated that, on average, a leaf was produced every 5.6, 7.2 and 8.1 days on the mainstem, primary and secondary tillers respectively. Thus it is clear that rate of leaf appearance on the different stems in barley was in the order - mainstem > primary tiller > secondary tiller; these results give no information on the relative rates of primordial initiation on the different stems, which will be discussed later in this chapter (page 76). There was no evidence that shading either the first or the second leaf had any effect after the initial perturbation, on the rate of leaf appearance on either the mainstem or any of the tillers.

(iv)/

(iv) Final leaf numbers per stem, and the dates of appearance of awns on mainstem and tillers.

Data on the final leaf number per stem are summarised in Table 3.5. There was no evidence that either of the shade treatments affected final leaf number per stem, although there were significant differences between the various tillers arising from different nodes, and between the mainstem and tillers. Using averaged values from the three treatments for leaf number per stem it was found that there were 2.1, 3.0 and 3.6 fewer leaves on tillers T1, T2 and T3 respectively than the mainstem. Values for T4 appeared to be similar to those for T3. Assuming a constant rate of leaf appearance of one leaf every 7.2 days on the primary tillers, as shown above (page 57), these results indicate that the period prior to the initiation of floral primordia in T1 was longer than that in T2 and T3 by approximately 6 and 11 days respectively.

The dates of awn appearance are also included in Table 3.5. Awns were visible on the mainstem 6 - 12 days before those on the primary tillers, but awning dates on the tillers T1, T2 and T3 were similar, usually within 3 - 4 days of each other, although in the control treatment and that involving shading the first leaf, awning was later on T1 than on either T2 or T3. This similarity is remarkable, and in view of the similar rates of leaf appearance, implying a similar growth rate in the tillers, and the progressively lower numbers of leaves per tiller at successively higher nodes, suggests that the transition from/

Table 3.5 Final leaf number per stem, and the number of days to awning (in parentheses) in control plants, and those having either the first or second leaf shaded; 95% confidence limits are indicated.

Stem	Control	Shaded	
		First Leaf	Second Leaf
M	10.1 ± 0.3 (64.8 ± 1.4)	9.9 ± 0.3 (66.6 ± 1.5)	10.1 ± 0.2 (65.4 ± 2.0)
T1	8.4 ± 0.4 (76.0 ± 3.3)	7.6 ± 0.4 (76.4 ± 2.1)	8.0 ± 1.8 (76.4 ± 6.7)
T2	7.1 ± 0.3 (72.4 ± 2.6)	6.9 ± 0.3 (72.9 ± 1.3)	7.1 ± 0.3 (72.8 ± 1.8)
T3	6.4 ± 0.4 (72.4 ± 1.5)	6.5 ± 0.4 (74.9 ± 2.6)	6.5 ± 0.4 (73.5 ± 2.8)
T4	6.0 ± 0.0 (77.3 ± 2.9)	—	6.5 ± 0.4 (78.8 ± 2.0)

from vegetative to reproductive development occurred at about the same time in primary tillers T1, T2 and T3. This possibility was investigated more fully in an experi-:ment to be described below (Section II(i) page 69). Awn appearance on T4 was significantly later than on tillers T1, T2 and T3 in the treatment involving shading the second leaf, and than T2 and T3 in the control. This suggests that the transition from vegetative to reproduc-:tive development was later in T4 than in tillers T1, T2 and T3.

Very few data were obtained on the dates of awning and final leaf numbers on the secondary tillers, but the indication was that awning occurred up to 10 days later, and that the final leaf number was about one less than on the parent primary tiller.

Shading either the first or the second leaf delayed the appearance of awns on the mainstem and all the tillers, although these differences were at a significant level only for the mainstem and T3 in the treatment involving shading the first leaf.

Thus awn appearance, which is delayed slightly on all stems by shading either the first or the second leaf, is approximately simultaneous in the primary tillers T1 - T3. This synchrony is associated with a decrease in the final numbers of leaves on stems at successively higher nodes.

(v) Yields of mainstem and tillers.

Dale et al. (1972) reported an overall decrease in total plant grain yield, due to a reduction in total tiller/

tiller yield, as a result of shading either the first or second leaf of barley. Shading did not, however, affect the yield of the mainstem. These findings were further examined by measuring grain yields of mature stems on the plants used in the leaf appearance studies described above, and the results are summarised in Table 3.6. It was again found that total plant yield was significantly reduced by shade treatment, and that there were no signi-:ficant differences in yields of each mainstem, primary or secondary tiller, in shaded plants compared to con-:trols. The difference in total yield of 200mg between control plants and those having their first leaves shaded could be explained by the fact (Table 3.3 page 51) that plants with their first leaves shaded had on average 1.0 less tillers at maturity than the controls. From the data in Table 3.6 it is clear that yield was greater on the mainstem than on a single primary tiller, which in turn yielded more than a single secondary tiller.

Similar effects to those on weights of grain were shown for numbers of grain borne, with shading either the first or second leaf having an adverse effect. The numbers of grains borne on the mainstem and primary tillers were unaffected by shading, but, for some unknown reason, plants having their second leaves shaded pro-:duced a smaller number of grains on their secondary tillers than the controls. These results indicate that shading either the first or second leaf had no effect on grain initiation and development on stems developing to maturity. The effect on total grain number can again be ascribed/

Table 3.6 Summary of data on grain yields of mainstem, primary and secondary tillers in control plants and those having either the first or second leaf shaded; 95% confidence limits are indicated.

	Control	Shaded	
		First Leaf	Second Leaf
Weights of grain (mg)			
Total Yield per plant (mg)	1920 ± 137	1720 ± 180	1734 ± 147
Total Yield of tillers (mg)	1348 ± 116	1105 ± 155	1144 ± 150
Yield per mainstem (mg)	572 ± 67	615 ± 95	590 ± 53
Yield per Primary Tiller (mg)	301 ± 33	312 ± 34	297 ± 37
Yield per Secondary Tiller (mg)	167 ± 61	198 ± 51	131 ± 76
Number of grains			
Total grains per plant	61.8 ± 3.7	53.4 ± 6.7	55.9 ± 4.8
Total grains from tillers	46.7 ± 3.6	36.7 ± 5.9	39.8 ± 4.6
Grains per mainstem	15.1 ± 1.6	16.8 ± 2.5	16.1 ± 1.3
Grains per Primary Tiller	10.2 ± 0.8	10.1 ± 1.1	10.3 ± 1.1
Grains per Secondary Tiller	6.8 ± 2.0	7.7 ± 1.3	4.8 ± 2.8
Grain weights (mg)			
Av. wt. per grain from whole plant (mg)	31.1 ± 1.5	32.4 ± 2.0	31.1 ± 2.0
Av. wt. per grain from mainstem (mg)	37.8 ± 1.4	36.7 ± 1.7	36.6 ± 2.2
Av. wt. per grain from 1^{o} tiller (mg)	28.8 ± 1.5	30.8 ± 1.3	28.9 ± 1.9
Av. wt. per grain from 2^{o} tiller (mg)	24.0 ± 3.3	25.8 ± 4.1	28.3 ± 7.5

ascribed to effects of shade on tiller number. As with the data on stem yield it is clear that there were differences between types of stem in the numbers of grains borne on each stem, with the order being as follows:- mainstem > primary tiller > secondary tiller.

There were no differences when weights of individual grains borne on the same type of stem in the three treatments were compared, but the average weight of a grain borne on the mainstem was greater than that of one on a primary tiller, which was greater than that of one borne on a secondary tiller.

When yields of TC - T4 were examined (Table 3.7) in the control plants, it was evident that the grain weight and number of grains on each stem were similar for the tillers T1, T2 and T3, and that these tillers gave higher yields than either TC or T4. There were no differences in the averaged weight per grain on the different primary tillers.

Calculation of the percentage of total grain yield produced on the different stems in control plants showed that the mainstem made the greatest contribution, followed by T3, T2, T1, T4, TC, T1.P and T2.P.

Thus total yield was significantly affected by treatment involving shading either the first or second leaf, due to an adverse effect on the numbers of tillers developing. Differences in grain yield between the various types of stem have also been shown, although shading had no effect on the yield of the particular stems developing to maturity.

Knowing/

Table 3.7 Grain Yields of primary tillers in control plants. 95% confidence limits are indicated.

	TC		T1		T2		T3		T4	
Total grain weight per tiller (mg)	203 ±	144	299 ±	74	353 ±	65	373 ±	40	219 ±	66
Total grains per tiller	7.3 ±	1.5	10.0 ±	2.0	11.4 ±	1.8	12.2 ±	1.1	8.6 ±	1.9
Av. wt. per grain on tiller (mg)	27.3 ±	15.0	29.5 ±	3.0	30.8 ±	1.5	30.5 ±	1.2	25.0 ±	3.4

N.B. The high values of the confidence limits for TC are due to the small number of TC tillers bearing grain.

Knowing the average number of leaves per stem (Table 3.5) and the final grain yield per stem (Table 3.6) a calculation was made to determine the grain yield per leaf produced on each stem; yields for the mainstem, T1, T2, T3 and T4 were 57, 36, 50, 58 and 37 mg respectively, and show no clear trends. Rawson (1971) showed a correlation between stem yield and stem dry weight for mainstem and tillers in wheat, and assuming a close relationship between stem dry weight and the number of leaves produced, the results just given are consistent with Rawson's findings.

(vi) Comparison of the growth of barley in 20/20°C and 20/17°C temperature regimes.

The results just given in sub-sections (i) to (v) have referred entirely to data obtained from plants grown throughout at 20°C. Control and treated plants were also grown in a regime of 20°C during the day and 17°C at night for reasons already discussed (page 46); the data obtained are summarised in Tables 1 - 7 of Appendix A. Except where specifically mentioned in the text below similar results to those already discussed for the 20/20°C regime were found for plants grown in the alternating temperature regime.

Initially dates of appearance of mainstem leaves were similar in both regimes, but later there was a slight delay in leaf appearance in the 20/17°C regime (App. A Table 1 cf. Table 3.1), so that the tenth leaf in the control plants and those having their first or second leaves shaded appeared later by 2.1, 2.8 and 0.4 days respectively/

respectively than in the corresponding treatments in the 20/20°C regime. For shade and control treatments the average slope of the regression lines for rate of leaf appearance on mainstem, primary and secondary tillers were 0.168, 0.133 and 0.117 in the 20/17°C regime compared to 0.178, 0.139 and 0.124 respectively in the 20/20°C regime. Although leaf appearance was marginally slower in the 20/17°C than in the 20/20°C regime, the final leaf number was slightly, but significantly, higher, 10.7 compared to 10.1 for control plants (App. A Table 5 cf. Table 3.5), and this indication of increased vegetative growth was shown also in the numbers of tillers appearing on each plant, 8.7 compared to 7.1 in control plants (App. A Table 3 cf. Table 3.3). However, the proportions of tillers surviving to maturity were smaller in the 20/17°C regime, 0.49 compared to 0.66 in the control treatment, so that there were in fact fewer tillers on each plant yielding grain in alternating than in constant temperatures. The proportions of visible tillers surviving to maturity in plants having their first or second leaves shaded, 0.49 and 0.40 respectively, were similar to that found in the control plants, and less than those for corresponding treatments in the 20/20°C regime.

Data on grain yield per stem showed similar results in both the 20/17°C and the 20/20°C regimes (App. A Table 6 and Table 3.6), except for three differences. Firstly, in the 20/17°C regime plants having their second leaves shaded yielded less grain than those having their first leaves shaded, due to a decrease in the total yield of the/

the tillers, although the reason for the poor yield of these plants is unknown. Secondly, the averaged weight of each grain on the various stems did not show clear differences, as found in the 20/20°C regime, in which grains borne on the mainstem were heavier than those borne on the primary tillers, which in turn were heavier than those on the secondaries. The third difference in grain yield in the two regimes was that yields per stem, and per plant, were lower in the 20/17°C than in the 20/20°C regime.

To summarise, growth of plants in the two regimes showed no major differences, although plants in the alternating temperature regime showed greater vegetative growth than those in the constant temperature, while the grain yield from mature stems, and the proportion of visible tillers surviving to maturity were smaller in the former than in the latter conditions.

(vii) Discussion

Data on the delay in leaf appearance due to leaf shade treatment are similar to those of Dale et al. (1972) and of Porter. The appearance of the second leaf is unaffected by treatment involving shading the first leaf, since the seedling continues to be dependent upon endosperm reserves until about day 8 after planting (Dale and Felippe, 1972), with the first leaf becoming maximally active photosynthetically from about this time in control conditions (Dale, 1972; Blenkinsop, 1974). Over the period day 8 - 11 the barley plant is totally dependent on photosynthesis in the first leaf for carbon assimilation, since/

since endosperm reserves are by then exhausted, and the second leaf does not start contributing significantly to plant dry matter increase until about day 12 (Dale and Felippe, 1972). Shading the first leaf prevents photo-:synthesis over this period and until the second leaf is active there is little carbon fixation by the plant, and a severe check in growth results. A less serious effect is seen for plants in which the second leaf is shaded, since these always have a supply of assimilated carbon, either from the first leaf over the period day 8 - 17, or from the third leaf from about day 17 onwards.

Although shade treatments have a persistent, long-:term effect in delaying the time of leaf appearance it is interesting that the rate of leaf appearance is unaltered, indicating that, as shown previously by Dale et al. (1972), the pattern of normal plant development is not changed by shading. Thus, the main effects of shading the first or, to a lesser extent, the second leaf are on developmental processes such as tiller bud initiation occurring during the period of shading. It is, however, clear that tillers capable of developing to maturity show no evidence of inhibited growth in terms of rates of leaf appearance or grain yield as a result of shade treatment. This implies that tillers become at least partially independent of the mainstem at a certain stage in plant development.

Plants used in the present experiments gave lower yields, in terms both of the numbers of grains, and grain weight, than those described by Dale et al. (1972), although/

although the grain used was from the same batch and growth conditions were regulated as far as possible to be similar; there is no obvious explanation for these differences. However, a similar effect of shading the first leaf in significantly reducing tiller yield was found, although treatment involving shading the second leaf caused a larger reduction in yield than that found by Dale et al. (1972).

II DEVELOPMENT OF MAINSTEM AND TILLER APICES

Results of experiments described in the previous section (page 58) showed synchrony in the dates of awning of the primary tillers T1 - T3, at about 6 - 12 days after the appearance of awns on the mainstem, and it was therefore of interest to investigate firstly, whether or not there was a synchronous transition from vegetative to floral apical development in these tillers, and, secondly, whether or not transition to the reproductive state occurred at different times in mainstem and tillers.

Other experiments to be described in this section further investigated differences in growth between the mainstem and tillers; firstly in terms of increasing apical dome size, and secondly, with respect to rates of primordial initiation.

(i) Time of transition from vegetative to floral development on mainstem and tillers.

Samples of at least 5, and usually 9, plants were dissected every second day from day 18 to day 34, and the stage of apical development in mainstem and tillers observed, and classified according to the scheme used by/

by Aspinall and Paleg (1963). The range of stages of apical development found at each harvest time is shown in Table 3.8.

By day 18 all mainstem apices were elongating, and some already had double-ridge structures present, whereas the apices of tillers TC - T3 showed no evidence of elongation at this time. Double-ridge structures were first visible on the T1 apex on day 24, and on TC, T2 and T3 on day 26, so that transition to floral development in these tillers occurred over a short period of about 2 - 4 days, similar to that over which awns became visible on primary tillers (Table 3.5, page 59). The difference in the dates of appearance of double-ridge structures on the apices of the mainstem and primary tillers was approximately 6 - 8 days, a slightly shorter time than that between mainstem and primary tiller awn appearance dates of about 6 - 12 days. This is compatible with the slower rate of leaf appearance on the tillers than on the mainstem.

The data show that apical development in TC was very variable, with some tiller apices showing no sign of apical elongation or double-ridge formation on day 34, whereas in others floral primordia with glume and lemma initials were visible at this time. It would appear that a proportion of TC tillers become moribund after only a short period of growth, although those tillers which continue to grow appear to develop double-ridge structures at about the same time as the primary tillers T1 - T3.

Data obtained in another experiment, investigating the/

Table 3.8 The ranges of stages of apical development in mainstem and primary tillers found in samples of control plants over the period day 18 - 34.

Day of harvest	M	TC	T1	T2	T3
18	2 - 3	1	1	1	1
20	3	1	1 - 2	1	1
22	3	1 - 2	2	2	1 - 2
24	3 - 4	1 - 2	2 - 3	2	2
26	5 - 6	1 - 3	3	3	2 - 3
28	5 - 7	1 - 3	3 - 4	3 - 4	3
30	6 - 7	1 - 4	3 - 6	3 - 5	2 - 3
32	7 - 8	1 - 3	5 - 6	4 - 5	3 - 4
34	9	1 - 5	5 - 7	4 - 7	4 - 6

Classification of stage of apical development according to the scheme of Aspinall and Paleg (1963), in which:-

Stage 1 = Single ridge on apex, apex not elongating

2 = " " " " , apex elongating

3 = Double ridge on side of apex

4 = Upper ridge enlarging

5 = Lateral spikelets visible as simple mounds

6 = Glume initials visible

7 = Lemma initials visible

8 = Stamen initials visible

9 = Awn initials visible

10 = Awns longer than spikelets

11 = Anthesis

the rate of primordial initiation, confirmed a delay of 6 - 8 days between the dates of appearance of double-ridge structures on the mainstem and primary tillers, and again showed a more or less synchronous transition to floral development on tillers T1 - T3.

It can be concluded that apices on tillers T1 - T3, and those on TC tillers which do not become moribund, respond to a floral stimulus in a synchronous fashion, and that development continues at a similar rate in primary tillers growing to maturity, resulting in the nearly synchronous appearance of awns. This point is further discussed below (page 118).

(ii) Apical dome size on mainstem and tillers.

A comparison of the size of the apical dome, the region above the youngest primordium (Felippe and Dale, 1973), of mainstem and tiller apices was carried out by comparing the dimensions on camera lucida tracings of apical domes exposed by dissection. Samples of 10 plants were dissected every second day from day 11 to day 25, and on day 28, and estimates of apical dome size obtained for the mainstem, and primary tillers T1 and T2. Determination of the areas of longitudinal sections through the middle of the apex, or direct measurement of dome volume using the squashing technique described by Sunderland and Brown (1956), and used by Felippe and Dale (1973), would have been alternative methods for the estimation of dome size, but would have been too laborious in view of the very large number of samples to be handled.

Comparison of the areas of the profiles of apical domes/

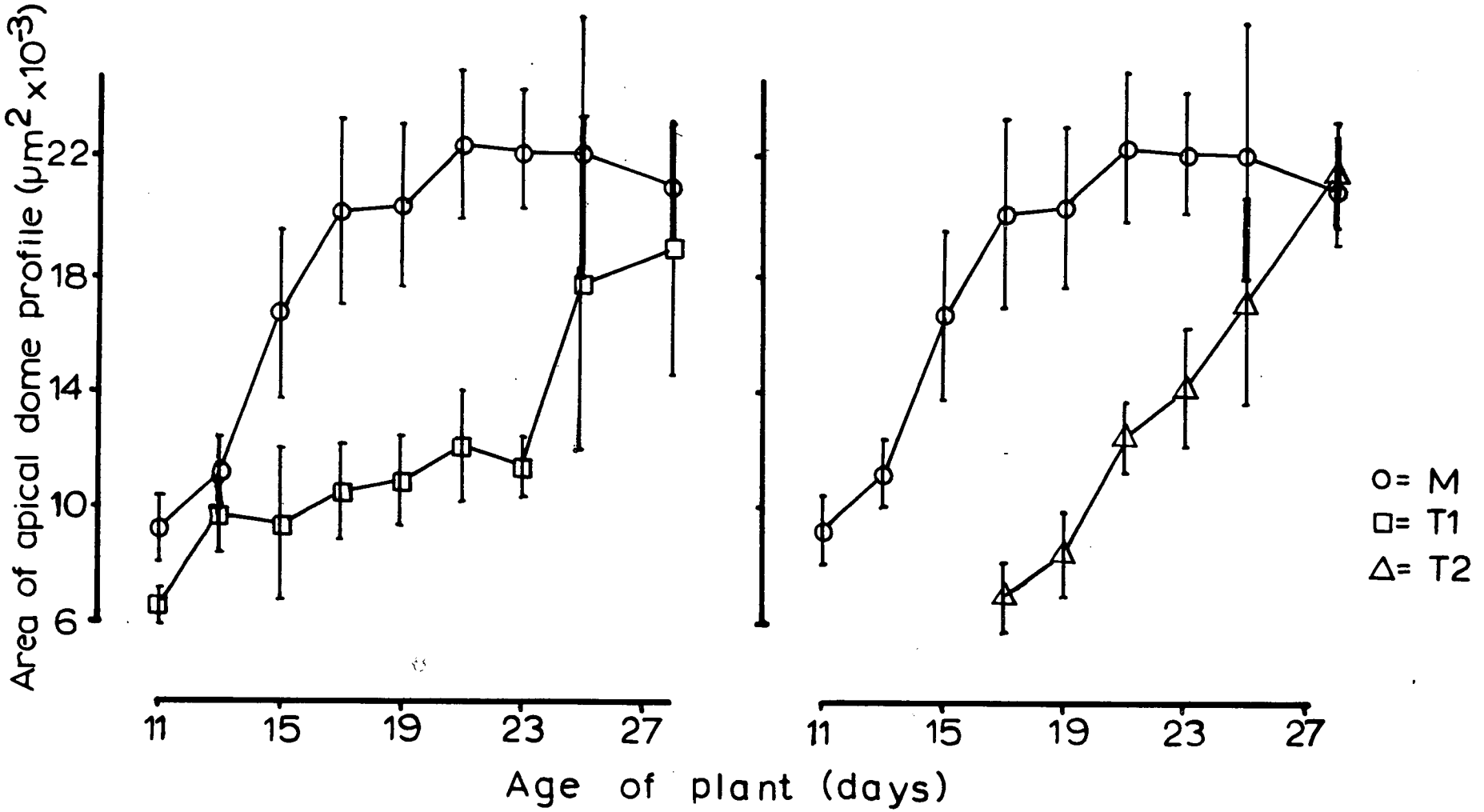
domes of mainstem and tiller apices are shown in Fig. 3.2, with the 95% confidence limits indicated for each sample. Initially the size of the mainstem apical dome was only slightly greater than that for T1, but the difference became more marked as the mainstem apex increased in size through elongation prior to the formation of double-ridge primordia. From day 21 it ceased to expand, and remained approximately constant in size up to the end of the experiment. From day 23 the T1 apical dome increased rapidly in size, and from day 25 it was not significantly different from the mainstem apical dome. Growth of the T2 apical dome was similar to that of T1, except that its rapid increase in size occurred from approximately day 19; by day 28 its size was also not significantly diffe-:rent from that of the mainstem. The apical domes of the two tillers showed some differences in size, especi-:ally early in the experiment, but on the later harvest days there were no significant differences between them.

The lengths of the apical domes of the mainstem and tillers followed a similar pattern of increasing size as that shown in Fig. 3.2 for increasing area of profile of the apical domes. On day 11 apical dome lengths were in the region of 75 - 100 μm, and by day 28 these had increased to 150 - 190 μm. By the end of the experiment there were no significant differences in apical dome lengths between the mainstem and tillers T1 and T2.

These results show that final apical dome size was similar in the mainstem and primary tillers T1 and T2, although the rapid increase in dome size preceding the appearance/

Figure 3.2 The increase in area of the profile of the apical dome with time, for apices of the mainstem and tillers T1 and T2; 95% confidence limits are indicated. Where there is an overlap in these limits between mainstem and tiller apical dome sizes the overlap is indicated by a broader line.

Fig 3.2


Area of apical dome profile ($\mu m^2 \times 10^{-3}$)
22
18
14
10
6
11
15
19
23
27
Age of plant (days)
o= M
□= T1
△= T2

appearance of floral primordia was later on the tillers.

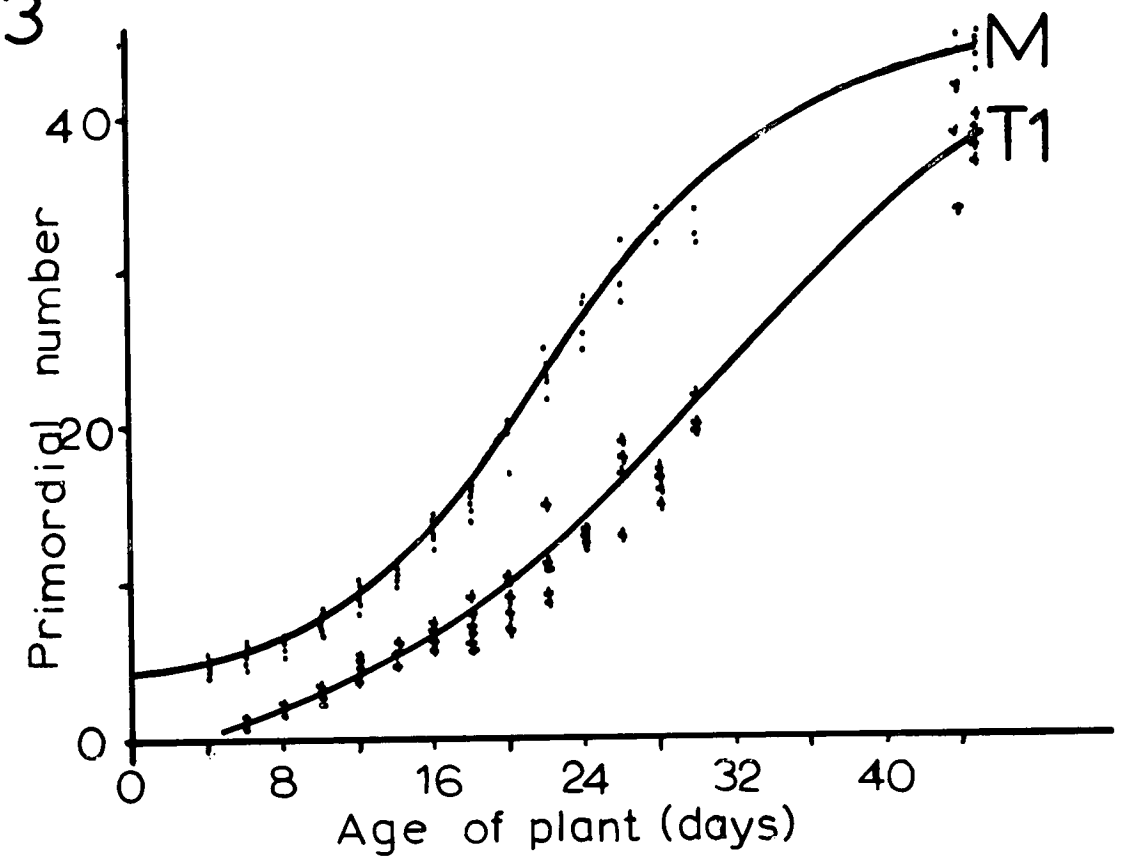
(iii) Rates of initiation of primordia on mainstem and tillers

To obtain data on the rates of initiation of primor-:dia on mainstem and tillers as a supplement to the results on rates of leaf appearance on the various stems (Section I(iii) page 52), samples of 5 - 7 plants were dissected every second day over the period day 4 to 30, and also on days 44 and 45. The total number of leaf and floral primordia, counting one spikelet as one floral primordium, as in Kirby (1973), present on each stem were counted, and the increase in primordial number with time is shown in Fig. 3.3. There were two reasons making it essential to use data on the initiation of the floral in addition to foliar primordia in order to compare rates of initiation on the mainstem and tillers. Firstly, the mainstem apex had completed initiation of foliar primor-:dia before some of the tillers had started primordial initiation; after germination the mainstem apex usually initiated only six foliar primordia, which together with the four present in the grain (Dale et al., 1972) gave a final leaf number of about 10 (see also Section I(iv), page 59). Secondly, it was sometimes difficult using the dissecting microscope to distinguish foliar and floral primordia in the early stages of their development.

The graph of the total number of leaf and floral spikelet primordia initiated on each stem against time was S-shaped (Fig. 3.3), and similar to that found by Lucas (1972) in wheat, cv. Triple Dirk. By plotting the total/

Figure 3.3 The increase in primordial number per stem for mainstem and tiller T1 with time.

Fig 3.3



total number of primordia on the mainstem against the tiller primordial number for each plant dissected, and using the data up to day 30, linear relationships were obtained (Fig. 3.4), and regression analysis was carried out on these. The correlation coefficients (Table 3.9) for the primary tillers T1 - T3 were very high, all above 0.95, indicating that the fitted straight lines account for over 90% of the variation in the data. The value for T4 of 0.79 was lower due probably to the fact that in the conditions of growth used T4 does not always appear above the leaf sheath, and therefore has rather variable development; nevertheless this value shows that the fitted line accounts for over 62% of the varia-:tion in the data. The correlation coefficients for the secondary tillers indicated that 65 - 81% of the variation in the data was accounted for by the fitted straight lines.

The values of the regression coefficients gave an indication of the rate of primordial initiation on the mainstem relative to that on each tiller. A value of one would have showed a similar rate on both types of stem, whereas values greater or less than one would have indicated respectively a faster or slower rate of primor-:dial initiation on the mainstem relative to that on the tiller. The regression coefficients were greater than 1.0 in all cases, being approximately 1.5 and over 2.0 for the primary and secondary tillers respectively (Table 3.9). There were no significant differences in the values of the regression coefficients for either the primary/

Figure 3.4 Numbers of primordia produced per tiller relative to the mainstem primordial number. Results for tillers T1, T1.P, T2 and T2.P are included in this figure. Results for T3, T4, T1.1 and T2.1 are included in Table 3.9.

Fig 3.4

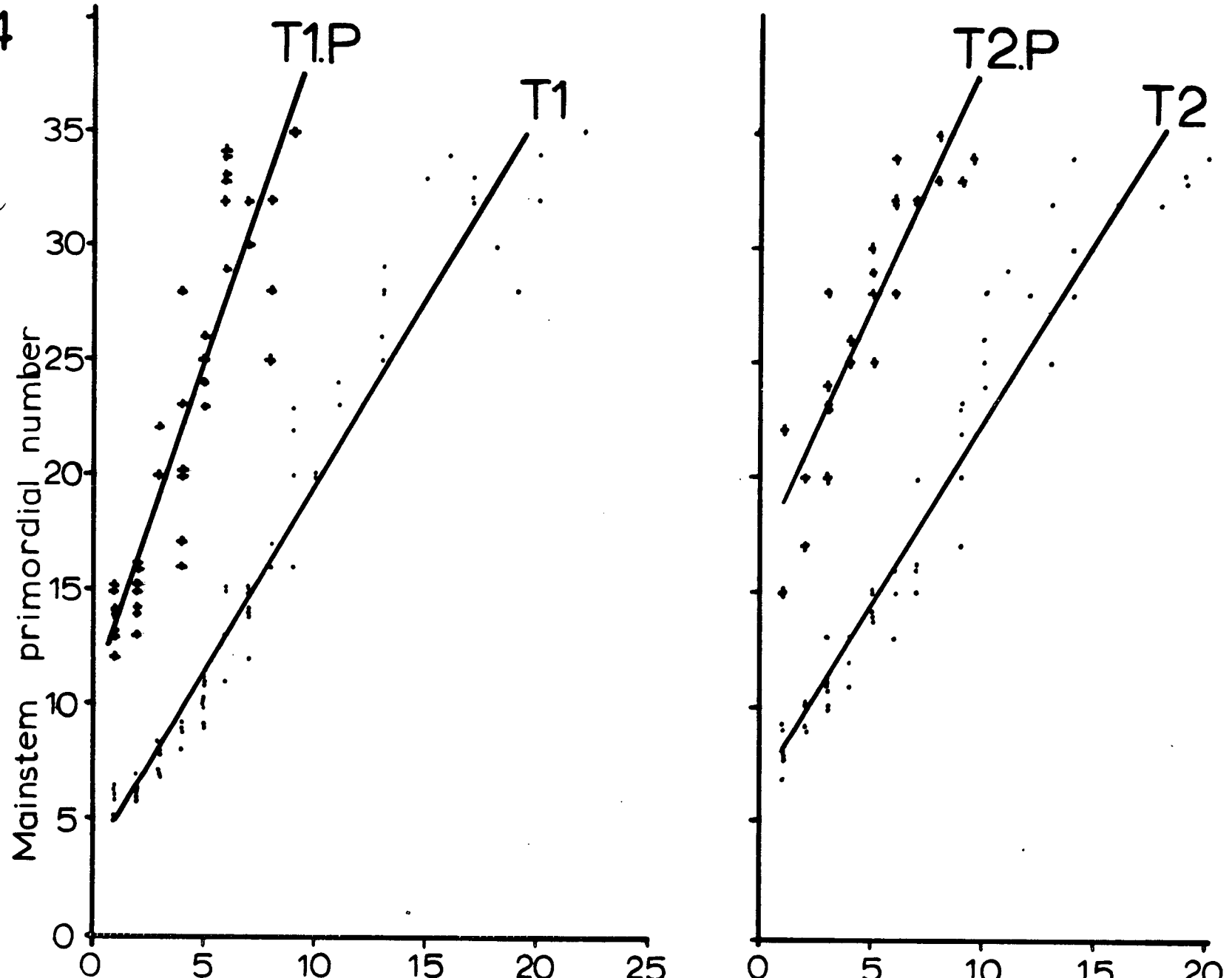


Table 3.9 Regression coefficients of the lines obtained by plotting the number of primordia on the mainstem against the number of primordia on a tiller. The coefficients indicate the rates of primordial initiation on the tillers relative to that on the mainstem. Correlation coefficients of the regression lines are also shown.

Comparison made	Regression Coefficient	Correlation Coefficient
Mainstem against		
T1	1.64	0.97
T2	1.58	0.97
T3	1.52	0.96
T4	1.27	0.79
T1.P	2.87	0.90
T1.1	2.59	0.85
T2.P	2.12	0.90
T2.1	2.34	0.81

primary tillers T1 - T4, or for the secondaries T1.P, T1.1, T2.P and T2.1 (Table 3.10). However both the primary tillers T1 and T2 had significantly higher rates of primordial initiation than their respective daughter secondary tillers. It is therefore clear that the rate of primordial initiation was greater on the mainstem than on the primary tillers which in turn had higher rates than the secondaries.

(iv) Discussion.

Dale et al. (1972) reported the presence of double-ridge structures on the mainstems of control plants on day 15 after planting, 3 days earlier than in the results given above. The reason for this difference is probably that in the earlier work apices were sectioned and observed microscopically, making it possible to identify double-ridge structures at an earlier stage of development than in the present work, where observations using the lower magnifications of a dissecting microscope depended upon the structures reaching a somewhat larger size for clear identification.

Felippe and Dale (1973) reported that on day 11 the length of the mainstem apical dome was about 70 μm, compared with the value of 100 μm obtained in the experiment just reported, and it appears that in the present work apical dome elongation continued longer than in the experiment described by Felippe and Dale. Several factors could contribute to these differences; firstly distortion due to using camera lucida drawings in the present work may have exaggerated apical dome size. Secondly,/

Table 3.10 Levels of significance in the comparisons of the rates of primordial initiation on primary and secondary tillers.

Comparison made	Level of significance
T1 v T2 v T3 v T4	n.s.
T1 v T1.P v T1.1	***
T2 v T2.P v T2.1	**
T1.P v T1.1	n.s.
T2.P v T2.1	n.s.
T1.P v T1.1 v T2.P v T2.1	n.s.

Key: n.s. = not significant at 5% level
** = significant at 1% level
*** = significant at 0.1% level

Secondly, Felippe and Dale examined apices which had been fixed, stained and sectioned for microscopical study, and may therefore have shrunk in size during processing for examination. Thirdly, very young primordia would have been easier to see using sectioned material than on camera lucida drawings of the whole apices, which may therefore have overestimated the size of the apical dome. However, none of these possible inaccuracies invalidates comparisons made between tiller and mainstem apices in the results just given, since the same method was used throughout the investigation. Felippe and Dale (1973) point out the difficulties in making comparisons of results obtained by different workers on apical dome size.

On day 26, at the time of transition to floral development in the tillers T1, T2 and T3, there were very great differences in the sizes of the tillers; T1 was a well-developed structure, fully emerged above the subtending leaf sheath; T2 was just large enough to have emerged, while T3 was a comparatively small structure which would only emerge above its subtending leaf sheath about 6 days later. An experiment to be described in the next section (page 87) showed that tiller dry weights on day 26 were approximately 96, 29 and 2.5 mg for T1, T2 and T3 respectively. Barley is photoperiodically sensitive (Borthwick, Hendricks and Parker, 1948; Aspinall, 1966), but it is unlikely that the leaves on T3 could have perceived the floral stimulus since this tiller was surrounded by the leaf sheaths of 3 mainstem leaves/

leaves on day 26. Thus it seems likely that the floral stimulus was perceived in the mainstem leaves, and translocated throughout the plant to affect apices in T1 - T3 more or less simultaneously, and hence to result in a nearly synchronous appearance of double-ridge structures in these tillers.

Differences between the various stems of the barley plant in the rates of appearance of leaves, and of initiation of primordia, must indicate that some form of control exists, through which the mainstem exerts an influence over the primary tillers, which in turn influence their daughter tillers, the secondaries. It is not clear from these results what causes this effect; possibilities include hormonal or nutritional effects, and these will be discussed later in this thesis (Chapter 6, page 232). There will also be further discussion of whether the effect is due to control by the mainstem of the tillers throughout growth, or to an initial difference between stems early in growth resulting in the long-term effect.

III SOME ASPECTS OF EARLY GROWTH OF TILLERS IN BARLEY

The lack of experimental data on early tiller bud development prior to emergence of the tiller from its subtending leaf sheath has already been referred to (page 17). Over the course of the project a number of experiments have been done in which growth in standard conditions of the tillers TC, T1 and T2, and the plant have been studied, and the overall results of this are described in this section.

As/

As shown previously (page 50) shading either the first or the second leaf reduces the numbers of tillers emerging from their subtending leaf sheaths, and two aspects of the shading effect are described here. These are firstly, the effect of shading the first leaf on the initiation, rather than emergence of tiller buds; and secondly, the effect of shading either the first or second leaf on early growth of tiller buds. Results of an experiment comparing the relative growth rates of tiller buds and mainstem leaves are also presented.

For all the experiments to be described in this section, plants were grown in standard nutrient conditions (Chapter 2, page 24); dissections were carried out and dry weights determined as described previously (page 30).

(i) Early growth of the whole plant and tiller buds TC, T1 and T2 in control conditions.

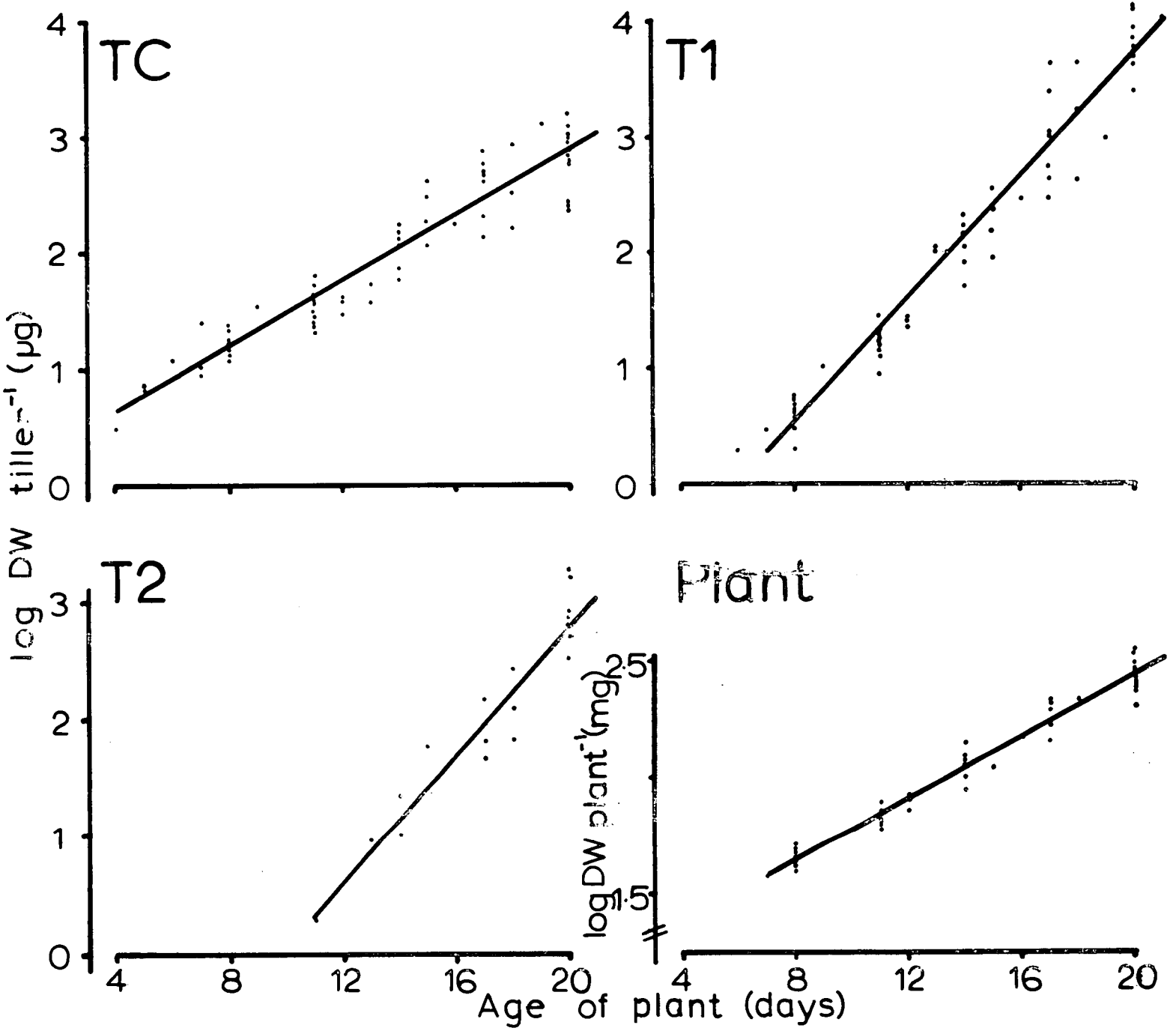
Tiller bud and whole plant dry weights were converted to $\log_{10}$ values, and the mean values calculated for each sample. Fig. 3.5 shows the growth curves for tillers TC, T1 and T2, and the plant over the first three weeks of plant growth; these data came from 16 similar experi-:ments. The linear relationships obtained show that both tillers and plant increased in weight exponentially, although the relative growth rate of the plant was lower than that for any of the tillers; between days 10 and 20 relative growth rates for the plant and tillers TC, T1 and T2 were 0.15, 0.32, 0.62 and 0.63 gg^{-1} day^{-1} respec-:tively. The regression coefficient of TC was signifi-:cantly lower ($p = 0.001$) than that or either T1 or T2, but/

Figure 3.5 Linear regressions indicating rates of dry weight increase for tillers (μg) TC, T1 and T2, and the whole plant (mg) over the first three weeks of growth using all available results from experiments in which plants were grown in control conditions.

Details of the linear regressions are as follows:-

	r	n	equation
TC	0.951	64	Y = 0.0942 + 0.1398X
T1	0.974	60	Y = -1.6153 + 0.2673X
T2	0.959	18	Y = -2.6567 + 0.2725X
Whole plant	0.987	43	Y = 1.1089 + 0.0667X

Fig 3.5


TC
T1
T2
Plant
log DW tiller^{-1} (µg)
log DW plant^{-1} (mg)
Age of plant (days)
0
1
2
3
4
1.5
2.5
4
8
12
16
20

but no difference was found between the latter tillers. It can be seen from the graphs that at the time of plant-:ing TC was heavier than T1 and that it was only from about day 13 - 14 that T1 became larger than TC.

The coefficients of variation of tillers TC - T3 were calculated from the data in one experiment, and are shown in Table 3.11. There was a fundamental difference between TC and the other tillers, in that the values of the coefficient for TC increased over the time period investigated, from about 7% on day 8 to over 30% on day 32, whereas values for the other tillers, T1 - T3 re-:mained approximately constant, or even decreased slightly. With one exception the value of the coefficient on a particular day was greater for T3 than for T2, and on all occasions the value for T2 was greater than that for T1. The increasing variability in size of TC is consi-:dered to be important and will be discussed further later (page 103). The fact that for the other tillers the values of the coefficients of variation are in the order T3 > T2 > T1 is probably associated with increasing variability in initiation of the later formed tillers; this lack of synchrony leading to a population of tiller buds whose physiological age was less closely related to chronological age at harvesting.

(ii) The effect of shading the first or second mainstem leaf on early tiller development.

Shading either the first or second mainstem leaf in barley has already been shown to cause a significant check in plant growth (page 48); treatment does not, however,/

Table 3.11 Changes in the coefficients of variation as percentages of $\log_{10}$ dry weights of tillers TC - T3 with increasing plant age.

Age of plant (days)	TC	T1	T2	T3
5	14.1			
8	7.0	6.3		
11	13.9	10.9		
14	15.4	9.1	9.3	
17	18.1	4.7	6.5	14.4
20	20.7	2.7	11.6	7.9
23	29.1	2.5	4.3	10.1
26	28.2	1.8	5.0	10.8
29	32.7	2.1	4.0	15.1
32	31.3	1.8	6.6	10.8

however, affect the pattern of growth of the mainstem, but does reduce both the numbers of tillers emerging from their subtending leaf sheaths, and the final number of grain-bearing tillers per plant. This reduction in tiller development through shading must have resulted from an inhibition either of tiller initiation, or of early tiller growth, or a combination of both effects. Experiments were carried out to try to distinguish these possibilities.

(a) The effect of shading the first leaf on tiller initiation.

The experiment to investigate the effect of shading the first leaf on tiller initiation involved dissection of 6 plants each of the control and treated plants on day 27 after planting. Day 27 is about 3 weeks before the emergence of the last formed tillers; assuming that, as for primary tillers, development from initiation to emergence takes about 3 weeks, it would be expected that tiller initiation in the plant was completed by day 27. The position of each tiller bud visible under the dissecting microscope after dissection was noted, and the numbers of primary, secondary and higher order, and total tillers initiated per plant by day 27 are shown in Table 3.12. Shading the first leaf had no significant effect on the number of primary tillers initiated, but there was a significant, 40% reduction in the number of higher order tillers initiated in shaded plants.

These data are in agreement with the observed reduction in higher order tillers visible in the yield study/

Table 3.12 Numbers of primary, higher order, and total tillers initiated per plant by day 27 after planting in control plants, and those having the first leaf shaded; 95% confidence limits are indicated.

	Control	Shaded first leaf
Primary	4.5 ± 0.6	4.3 ± 0.5
Higher order	6.8 ± 1.8	4.0 ± 1.0
Total	11.3 ± 2.2	8.3 ± 1.1

study discussed earlier (section I(v), page 60). The lack of effect of shading the first leaf on the number of primary tillers initiated suggests that the observed reduction in primary tillers emerging and growing to maturity on shaded plants (Table 3.3, page 51) was due to an adverse effect on early tiller growth.

(b) The effect of shading either the first or the second leaf of barley on the early growth of tillers TC, T1 and T2.

Two experiments were carried out to investigate the effects of shading on early tiller growth. In the first, control and treated plants having their first leaves shaded were dissected every second day from day 7 to 17, with 10 plants comprising each sample. In the second experiment plants treated with either their first or second leaf shaded were compared with controls, and samples of 8 plants of each treatment were dissected every 3 days from day 8 to 23. Results from these expe-:riments are presented graphically (Fig. 3.6), and also in terms of relative growth rates (Table 3.13); these data are taken together in the following discussion.

As shown in previous results (Fig. 3.5, page 89) all the tiller buds TC, T1 and T2 increased in dry weight exponentially in unshaded, control plants, up to day 23 (Fig. 3.6). Treatment involving shading the first leaf allowed only slight growth of tiller buds up to day 13 or 14, and on those days in the first and second experi-:ments respectively, bud sizes were about a tenth of those in the control plants.

Relative growth rates for tillers on shaded plants were/

Figure 3.6 Increase in dry weight of tillers (μg) TC (A, C), T1 (B, D) and T2 (E), and the whole plant (mg) (F), grown under control conditions, or with either the first or second leaf shaded. A - B and C - F refer to two separate experiments.

Fig 3.6

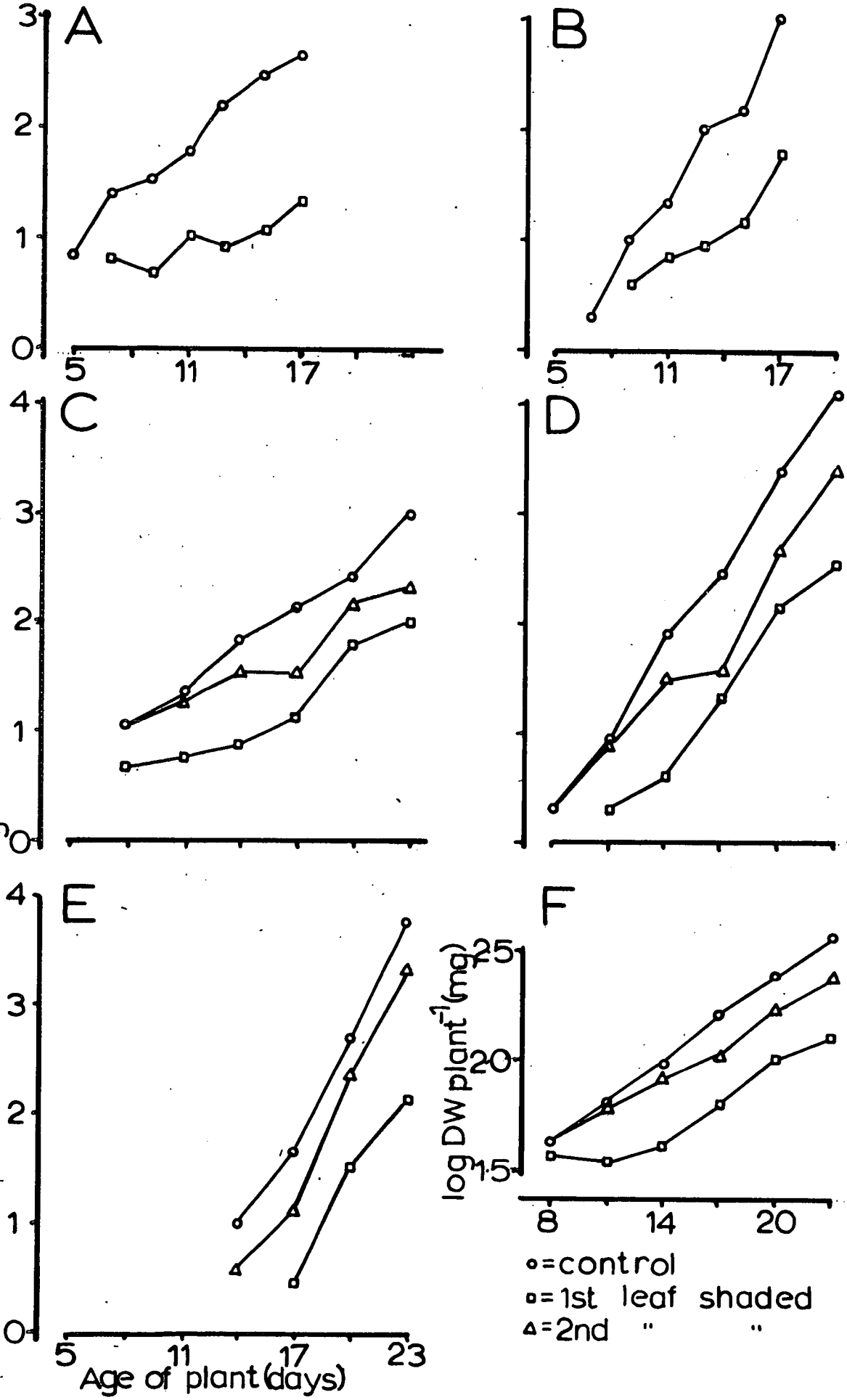

A
B
C
D
E
F
3
2
1
0
4
5
11
17
23
8
14
20
25
log DW plant^{-1} (mg)
Age of plant (days)
o = control
□ = 1st leaf shaded
Δ = 2nd " "

Table 3.13 Relative growth rates (gg^{-1} day^{-1}) of tillers and whole plants in control plants and those having either the first or second leaf shaded. Figures in parentheses indicate the percentage of the relevant control value.

Experiment	Period over which R measured(days)	Structure for which R measured	Control	Shaded	
				First Leaf	Second Leaf
I	7 - 17	TC	0.29	0.12(41)	-
		T1	0.62	0.41(66)	-
II	8 - 17	TC	0.27	0.12(44)	-
		T1	0.55	0.34(62)	-
		Whole Plant	0.15	0.06(40)	0.10(67)
II	17 - 23	TC	0.33	0.33(100)	0.31(94)
		T1	0.64	0.48(75)	0.71(>100)
		T2	0.81	0.64(79)	0.84(>100)
		Whole Plant	0.13	0.12(92)	0.13(100)

were less than for those of untreated controls (Table 3.13) over the early part of both experiments, but differences became less marked over the period day 17 - 23 in the second experiment. These trends are clearly seen when relative growth rates for tillers on shaded plants are calculated as percentages of the control values (Table 3.13). In both experiments for plants having their first leaves shaded the percentage values for TC, up to day 17, were smaller than those for T1. This indicates that growth of TC was reduced more than that of T1 through treatment involving shading the first leaf.

Shading the second leaf was found to reduce the absolute growth of all the tillers TC, T1 and T2 (Fig. 3.6), but to a smaller extent than in plants with their first leaves shaded. There was an apparent inhibition of growth of TC and T1 over the period day 14 - 17, although T2 was unaffected over this period; this observation is referred to again later (page 105). When relative growth rates over the period day 17 - 23 were studied (Table 3.13) it was found that both T1 and T2 showed slightly higher values than those for control plants, although whether the differences are significant biologically is unknown.

Growth of the whole plant was also affected by shade, especially treatment of the first leaf, which caused a delay in the start of exponential growth from day 8 to day 14 (Fig. 3.6). Shading the second leaf had only a slight effect up to day 14, and it appears that the greatest effect was over the period day 14 - 17.

From/

From day 17 to the end of the experiment the rates of growth of control plants and those having their first or second leaves shaded were similar, although the absolute weights on day 23 were smaller in the treated plants.

Thus, growth of both the plant and the tillers was affected by shading either the first or the second leaf, although the former treatment had larger effects than the latter. Tiller TC was relatively more affected than T1 through shading the first leaf, and growth of both TC and T1 was inhibited to a greater extent than that of T2 over the period day 14 - 17 when the second leaf was shaded. These data indicate that the main effect of shading is on early development, rather than initiation, of the primary tillers.

(iii) A comparison of the rates of growth of leaves and tillers.

As discussed in the Introduction (Chapter 1, page 14) there are a number of reports that a tiller is more closely associated with the mainstem leaf at the next younger node than the leaf in whose axil it is subtended. It was thought that a study of the early rates of growth of both leaves and tillers might give further information on this association; and an experiment, in which dry weights were obtained of the constituent parts of 12 plants dissected every 3 days over the period day 5 - 32 was carried out.

The growth patterns of tillers TC - T4 and leaves 1 - 5 are shown in Fig. 3.7, and the maximum relative growth rates for each organ given in Table 3.14. Tiller TC/

Figure 3.7 Time course of growth in dry weight (μg) of successive primary tillers and mainstem leaves. Arrows indicate the dates of appearance of tillers.

Fig 3.7

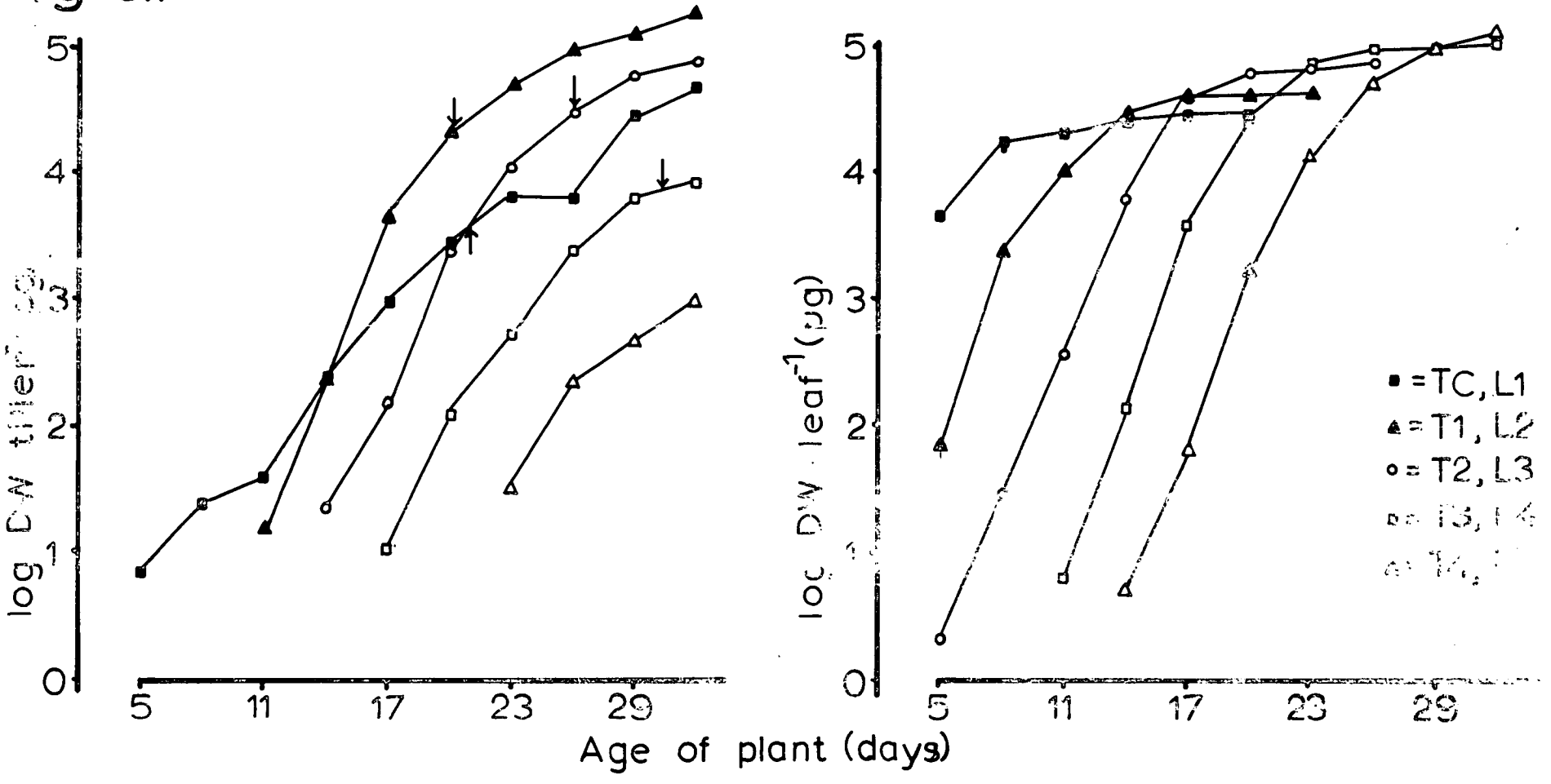

log DW tiller⁻¹ (µg)
log DW leaf⁻¹ (µg)
0
1
2
3
4
5
5
11
17
23
29
Age of plant (days)
■ = TC, L1
▲ = T1, L2
○ = T2, L3

Table 3.14 Maximum mean relative growth rates ($gg^{-1}day^{-1}$) for tillers and mainstem leaves. The 3 day period over which the value was obtained is shown in parenthesis.

TC	T1	T2	T3	T4
0.60 (11-14)	0.99 (14-17)	0.92 (17-20)	0.74 (17-20)	0.65 (23-26)

L2	L3	L4	L5
1.17 (5-8)	0.94 (11-14)	1.12 (14-17)	1.06 (17-20)

TC had the lowest maximum value of the tillers examined, and the tillers T1 - T4 had progressively lower maximum relative growth rates. The values of the maximum growth rates for leaves 2 - 5 were very similar, as also were the growth curves for these leaves (Fig. 3.7). Leaf 1 was already substantial in size by day 5 after planting, and over the duration of the experiment showed no period of rapid exponential growth as in the other leaves. In this experiment the rate of leaf appearance was faster than in the study described previously (section I(i), page 46), and leaf 9 appeared on day 42. Assuming a regular rate of leaf appearance over the duration of the experiment, and that the second leaf appeared on day 8 after planting, it follows that the dates of appearance of leaves 3, 4 and 5 were days 13, 18 and 23 respectively. As can be seen from Fig. 3.7 these dates correspond approximately with the cessation of exponential growth in the leaves. The maximum growth rates of the tiller buds were consistently found to be lower than those for the mainstem leaf most closely associated with it at the next higher node.

(iv) Discussion

Over the first three weeks of plant growth a lower rate of exponential growth is found in TC than in T1. This must indicate either that all TC tillers grow at a slower rate than T1 tillers, or that growth of TC is more variable than that of T1, with sufficient of the TC tillers having extremely low rates of growth to depress the overall mean values. Data on the coefficients of variation/

variation show a clear difference between the two classes of tillers over the time period investigated. The values of the coefficient for T1 remain approximately constant over the first three weeks of plant growth, showing that for this tiller variation within each sample does not change over this period. In contrast, the values of the coefficient for TC increase over the same period indicating increasing variation in tiller size within samples.

On days 14 and 32 samples of 11 tillers each of TC and T1 had dry weights as follows:-

Day 14:

TC (µg)	72	80	104	116	116	146	162	170	196	712	724
T1 (µg)	102	110	152	154	178	204	240	256	326	384	498

Day 32:

TC (mg)	026	040	041	046	056	430	860	900	900	920	1000
T1 (mg)	141	147	160	161	161	166	194	206	217	250	258

From these results it is clear that by day 32 the sample of TC tillers was showing a bimodal distribution in dry weights, with 6 tillers each weighing 40 - 100 mg, and 5 each weighing less than 1 mg. On the same day the sample of T1 tillers showed less variability, with all the tillers weighing 140 - 260 mg. That a considerable proportion of TC tillers becomes extremely slow growing, or moribund, over the first 3 - 4 weeks of growth is also indicated from results investigating apical development, which showed that some TC tillers never develop beyond the stage of apical elongation (Table 3.8, page 71).

Shade treatment of the first leaf has a greater effect than that of the second leaf on plant development over the first three weeks of growth since with the first leaf/

leaf shaded no carbon assimilation can occur in the plant until the second leaf starts photosynthesising, whereas photosynthesis is occurring in the first or the third, or both these leaves throughout the time the second leaf is shaded. Tiller TC is at a relatively greater disadvan-:tage than T1 when the first leaf is shaded since TC is more closely associated with the first than with the second leaf (Langer, 1972; see also page 214). In plants having their second leaves shaded growth of tillers TC and T1 is considerably reduced over the period day 14 - 17, when the second leaf would be expected to be photosynthesising at its maximum rate. However, it is interesting that T2, which develops in the axil of the second leaf, is much less affected and continues to grow over the period day 14 - 17, although in absolute terms T2 remains smaller in treated plants than in controls up to day 23.

IV SOME EFFECTS OF GRAIN AGEING

The problem of whether to use the same batch of grain for as much as possible of the experimental work in this project, or to use new stocks of grain each year has already been mentioned (Chapter 2, page 22). It was decided to use the same batch of grain harvested in 1970 for the majority of the experiments.

Over the course of the project two sets of experi-:mental results have been obtained in connection with effects of grain ageing: firstly, the effect of increa-:sing grain age on the rate of exponential increase in dry weight of the tillers and plant; and secondly, the effect of varying grain age on the numbers of leaves produced/

produced on each mainstem, and tillers produced per plant.

(i) The effect of grain age on the rate of increase in dry weight of the tillers and the whole plant.

Grain supplied by the Scottish Plant Breeding Institute, Pentlandfield, and harvested in 1970 was used throughout this experiment. It was stored for approximately 16 months prior to the first run of the experiment in January, 1972, and over a period of 26 months from that date a further 5 runs were carried out. In all the runs plants were grown in standard conditions in a controlled environment room, and samples harvested on days 8, 11, 14, 17 and 20. Linear regressions for the increase in log dry weight of the plant and of the tillers were calculated, and correlation and regression coefficients for all the runs are shown in Table 3.15.

With one exception correlation coefficients were all above 0.97, indicating that over 94% of the variation in each set of data was accounted for by the linear regression. Regression coefficients for each time of planting were compared with the appropriate value for the initial set. For the plant data the value for the 5 month set was significantly higher ($p = 0.05$) than that for the zero time set, but the other sets showed no statistically significant differences. Although values for neither of the tillers differed statistically from the relevant zero time value, TC values showed a general decrease with increasing grain age; the results for T1 were more irregular, and showed no such trend. There was a/

Table 3.15 Correlation and regression coefficients of the linear regression lines showing rates of growth of tillers, T1 and T2, and the whole plant, in sets of plants grown from the same batch of grain after varying lengths of storage. Grain aged for 16 months was used to produce plants in the first run of the experiment.

Time of grain storage after initial set planted (mths)	TC		T1		Whole Plant	
	Correlation Coeff.	Regression Coeff.	Correlation Coeff.	Regression Coeff.	Correlation Coeff.	Regression Coeff.
0	0.98	0.164	0.99	0.258	0.99	0.062
3	0.99	0.157	0.99	0.287	0.99	0.069
5	0.98	0.156	0.99	0.308	0.99	0.075
9	0.99	0.145	0.99	0.321	0.99	0.068
16	0.93	0.112	0.99	0.226	0.99	0.063
26	0.99	0.115	0.99	0.257	0.99	0.064

a 30% reduction in the value of the regression coefficient for the TC tiller in the 26 month set compared to the initial value, but no reductions for either T1 or the plant were found.

There is therefore no evidence of an effect of grain ageing on the rates of log dry weight increase of either the plant or tiller T1, but an effect on TC is suggested over the period of 26 months investigated. It is possible that this effect on TC is related to the lower vigour noted by Cannell (1969b) (see also page 87).

(ii) The effect of grain age on the numbers of leaves produced on the mainstem, and the total number of tillers produced per plant.

Measurement of the numbers of leaves per mainstem and tillers per plant gave an indication of the amount of vegetative growth which the plant supported. Batches of grain of varying age were generated during the project, and two experiments were carried out as follows:-

(a) A set of plants grown from grain harvested in 1969 was cultured in standard conditions in a controlled environment room and harvested in September, 1970. Grains from this harvest were planted in October, 1970 and cultured simultaneously with a set of plants grown from the 1969 grain, and used to study the rates of leaf appearance on mainstem and tillers in a temperature regime of 20°C during the day and 17°C at night. Grain from the September, 1970 harvest was also planted 4, 7, 10 and 19 months after harvest, in each case the sample size being 10 plants. In these runs the temperature was maintained at 20°C day and night; abnormal plant growth and electrical/

electrical power cuts prevented the completion of runs using grain aged for 13 and 16 months respectively. Thus, five different sets of plants were grown from the same batch of grain over a period of 19 months; leaf number on the mainstem and numbers of tillers produced per plant were measured to investigate whether or not grain age affected either of these aspects of plant development, and the results are shown in Table 3.16.

Plants grown from grain harvested one month previously had significantly higher numbers of tillers per plant than any of the sets grown from grain 4, 7, 10 or 19 months old. The value of 12.9 ± 1.7 was also higher than the value of 8.7 ± 1.6 obtained for the control plants; in this experiment control plants were grown from grain of the 1969 harvest from the Scottish Plant Breeding Institute, and cultured in a regime of 20°C during the day and 17°C at night (App. A, Table 3). Numbers of leaves on the mainstem were also highest in the set planted one month after harvest; there were significant differences when this value was compared with those of the sets planted 4 and 7 months after harvest, but the result did not reach a significant level when compared with the set planted 10 months after grain harvest.

It appears therefore that the grain recently harvested produced plants with more leaves per mainstem and tillers per plant than the sets from grain aged 4 months or more. However, there is slight uncertainty as to the validity of this effect, as the set grown from/

Table 3.16 Results from two experiments investigating the effects of grain ageing on plant vegetative growth.

Experiment 1. Numbers of leaves per mainstem and visible tillers per plant produced on plants grown at 3-:monthly intervals from the same batch of grain; 95% confidence limits are indicated. Numbers in parentheses indicate the coefficients of variation, as percentages, for the numbers of tillers per plant.

Time between grain harvest and planting (months)	Number of leaves per mainstem	Number of tillers per plant
1	12.2 ± 0.3	12.9 ± 1.7 (18.1)
4	11.2 ± 0.3	8.1 ± 1.2 (20.6)
7	10.9 ± 0.2	9.0 ± 1.7 (26.2)
10	11.6 ± 0.7	8.3 ± 1.1 (18.0)
19	—	8.8 ± 1.4 (22.6)
Control value	10.7 ± 0.3	8.7 ± 1.6 (25.4)

Experiment 2. Numbers of visible tillers per plant pro-:duced on plants grown simultaneously from batches of grain of varying age; 95% confidence limits are indicated. Numbers in parentheses indicate the coefficients of variation, as percentages.

Time between grain harvest and plan-:ting (months)	Numbers of tillers per plant
5	10.6 ± 2.0 (26.0)
8	12.1 ± 2.9 (33.8)
11	10.6 ± 3.2 (42.0)
19	8.8 ± 1.4 (22.6)

from grain aged one month was cultured in a different temperature regime from the other sets.

(b) Further batches of grain were generated by collecting the yields from the sets planted 4, 7 and 10 months after harvest. Ten plants from each of these batches of grain aged 11, 8 and 5 months respectively were grown simultaneously with the set originating from grain of the September, 1970 harvest, aged 19 months. Thus, four sets of plants were grown simultaneously from batches of grain of varying age produced in controlled environmental conditions. Numbers of tillers produced per plant were measured, and the results are included in Table 3.16.

No convincing differences were shown in the amount of tillering from plants grown from grain varying in age from 5 to 19 months. For some reason the grain produced after two generations of growth in the controlled environment room, i.e. the sets 5, 8 and 11 months old, gave plants showing greater variability in tiller number, as indicated by the values of the coefficient of variation, than the plants grown from grain developed on plants grown for only one generation in the controlled environment room, i.e. the plants grown from grain aged 19 months.

It can be concluded that over the period of time investigated the only effects of grain ageing found were firstly, the decreased numbers of leaves per mainstem and tillers per plant on plants grown from grain aged for 4 months or more; and, secondly, the decrease in the initial rate of dry weight increase in TC.

Although/

Although there is evidence of slight effects of grain ageing on plant and tiller growth it is felt that these results do not invalidate the argument given in Chapter 2 (page 22) favouring the use of a single batch of grain for as many experiments as possible in the project.

V DISCUSSION

Shading the first leaf of barley has a severe effect on tiller initiation and reduces the final number of grain-bearing stems on each plant, as shown by Dale *et al.* (1972), and confirmed in the present results. The absolute nutritional requirements of tiller buds must be very small initially, due to their small size relative to the rest of the plant, but shading the first leaf prevents even these requirements being met for tillers TC and T1, and the onset of exponential growth of these buds is delayed. However, those tillers growing to maturity in plants having either their first or second leaves shaded have exactly similar rates of leaf appearance to those in control plants, and eventually produce similar grain yields. From these results it seems that, provided the tiller's potential for growth is not reduced during the period of shade treatment, the tiller can continue to grow when sufficient assimilate becomes available; once it reaches a stage of development allowing it to grow more or less independently of the mainstem, its development is not further inhibited by shade treatment.

It is uncertain if the effect of shade treatment on tiller growth is direct, by causing a reduction in the amount/

amount of assimilate available to the growing regions of the plant; or indirect, involving, for instance, a reduc-:tion in root growth which could limit uptake of minerals such as the nitrate ion. Another indirect effect of shading might be in limiting nitrate reductase activity, which is known to be reduced in shaded conditions (Hage-:man and Flesher, 1960; Dale, Felippe and Marriott, 1974); this could result in insufficient organic nitrogen com-:pounds being available to support full tiller growth.

Tiller TC grows less rapidly than either T1 or T2, and also tends to be less vigorous (Cannell, 1969b). Some of the results given in this chapter, and a number of other observations, can be used to suggest reasons for the differences between these tillers. There is evidence (Bunting and Drennan, 1966; Langer, 1972; see also page 214) that a tiller is most closely associated with the leaf at the next younger node; thus TC is associated with the first leaf, and T1 and T2 with the second and third leaves respectively. Leaf 3 is larger than leaf 2, which in turn is larger than leaf 1, although the differences are relatively small. The other main difference between these leaves is that the first leaf is the plant's only photosynthesising organ over a consi-:derable period of time, whereas at least one other leaf is active in assimilation when later formed leaves are functioning. If the initial growth of a tiller bud is dependent simply on its association with the leaf from the node above, it would be expected that differences in the early rates of exponential dry weight increase in tillers/

tillers TC, T1 and T2 would be only slight. However, the rate of increase in dry weight is significantly lower in TC than in either T1 or T2, but there is no difference between the latter two tillers. From these considerations it appears that some factor other than the association with the leaf above must affect growth of young tiller buds.

TC develops in the axil of the coleoptile, and T1 and T2 in the axils of leaves 1 and 2 respectively. The coleoptile is a non-photosynthesising structure, which protects the apex and young leaves as they grow towards the soil surface after germination; coleoptile growth is completed by day 8, giving a final dry weight of approximately 1 - 2 mg. The first leaf expands over a longer period than the coleoptile, up to day 8 - 9, and has a mature dry weight of 15 - 20 mg (Blenkinsop, 1974). The second leaf reaches its maximum size about day 12 - 13, and has a final dry weight somewhat greater than that of the first leaf (Felippe and Dale, 1973). Thus the leaves in whose axils T1 and T2 are positioned develop over a longer period than the coleoptile. It seems possible that there is some association between a tiller bud and the structure in whose axil the tiller develops, as a result of which materials being transported into the leaf/coleoptile during their development diffuse into the young tiller bud. Thus tillers T1 and T2, developing in the axils of the larger, longer developing leaves 1 and 2 would receive more assimilate than TC, developing adjacent to the small, rapidly developed/

developed coleoptile. An association between a leaf/ :coleoptile need not necessarily involve vascular connec- :tion, but could be of great importance before the tiller develops any vascular connections with other parts of the plant.

Rawson (1971) showed that in wheat the secondary tillers in the axils of prophylls, structures on the tillers corresponding to the mainstem's coleoptile, on the primary tillers were poorly developed compared to those in the axils of the true leaves. These data are consistent with the idea that the organ in whose axil the tiller develops may influence early tiller bud growth, since the prophyll is a considerably smaller structure than the true leaves on the tillers. During its expansion therefore, the prophyll must be a less powerful sink for nutrients than the leaves and the growth of the bud in the axil of the prophyll may be limited due to its association prior to the formation of vascular connections with the prophyll rather than a true leaf.

Another possible cause for the differences in the growth pattern of TC and T1 is a difference observed in their structure. Over the whole project a large number of plants have been dissected, and it is clear that the connection between TC and the mainstem is less strong than that between T1 and the mainstem. TC is a somewhat narrower structure than T1, and the area of contact between the base of TC and the adjacent mainstem is less than that for T1. It may be therefore that there is less vascular connection with the mainstem for TC than for T1; the/

the smaller area of contact could also limit diffusion of materials into TC. It is not known if there are structural differences between T1.P and T1.1 similar to those noticed between TC and T1.

These observations suggest two possible causes for the poorer growth of TC than T1; firstly, its position in the axil of the coleoptile rather than a true leaf, or, secondly, its smaller vascular connection with the mainstem. These possibilities will be investigated further during the rest of this thesis.

Differences in the rates of primordial initiation and leaf appearance between the mainstem, primary and secondary tillers have not previously been shown and are of some interest. Rates of both primordial initiation and leaf appearance were shown to be remarkably similar for different stems within each type, with no clear differences between the primary tillers T1, T2 and T3, or between the secondaries T1.P, T1.1, T2.P and T2.1; the difference between stem types must therefore be an effect of stem hierarchy. It is known that tillers eventually become more or less independent of the mainstem (Bunting and Drennan, 1966), having their own photosynthesising leaves and adventitious root system, although connection is maintained with the mainstem throughout the plant's development. However, it is uncertain whether the differences in rates of primordial initiation and leaf appearance on the various stem types are due to some form of inhibition from the stem on which they are borne, or are an inherent property of the tiller/

tiller itself.

It is possible that the photosynthetic leaf area on each stem controls the rate of leaf emergence on that stem; leaves on the tillers appear to be smaller than those on the mainstem, and this could be the cause of the lower rate of leaf emergence on the tillers than on the mainstem. However, this difference between stems cannot be the cause of differences in primordial initiation rate between mainstem and tillers over the first 2 - 3 weeks of tiller bud development up to the time of tiller emergence; over this period the tiller must be com-:pletely dependent on the mainstem for assimilates and mineral nutrients. It seems probable therefore that the differences in the rates of primordial initiation on the various stems must be due to some influence of the mainstem over the primary tillers, and of the pri-:maries over the secondaries. The nature of such an influence is uncertain, and the data available give no indication of whether it could be a stimulatory influence of greater magnitude in the mainstem than in the tillers, or an inhibitory influence present to a greater extent in the primary tiller than in the mainstem. Such an effect might also result from the availability of neces-:sary nutrient or assimilate, with the mainstem receiving a better supply than the tillers. Another possibility was that apical dome size might be different on mainstem and tillers, and that this could be the cause of diffe-:rences in rates of leaf appearance on the various tillers. These possibilities will be further discussed later/

later (page 232).

The synchrony shown in tiller apical development, both in the transition of the apices from vegetative to floral development, and in the appearance of awns about 50 days later is also of interest. At the time of trans-:ition of the apex to floral development little elonga-:tion of the stem has occurred, and therefore the tiller buds are positioned close to each other and to the junc-:tions between leaves and mainstem. The mainstem apex receives the floral stimulus, presumably perceived in the leaves, and about 5 days later the tillers also become floral in their development. As far as is known synchrony of primary tillers T1 - T3 in their transition to floral development has not previously been shown.

None of the tillers which yielded grain had less than 6 leaves, and it would therefore appear that a minimum number of leaves must be present on each tiller to allow it to develop to maturity. In the experiment in which the rate of primordial initiation was studied it was found that on day 26, the day by which floral structures were visible on tillers T1 - T3, the numbers of primordia on tillers T1, T2, T3 and T4 averaged 16.8, 13.5, 11.3 and 4.0 respectively. T4 did not often develop to maturity and it seems that an average of 4.0 primordia on the apex initiated by the time double-ridge structures are first visible is insufficient to allow the tiller to develop to maturity. Other data indicate that the mainstem exerts a control over the initiation of primary tillers, since T5 was rarely initiated.

Evidence/

Evidence to be presented later in this thesis (page 213) suggests that T1, T2 and T3 are initiated on days 2, 6 and 10 respectively. If this rate continued initiation of T4 and T5 would be expected on days 14 and 18 respec-:tively. By day 18 the mainstem has received the floral stimulus, and it therefore seems probable that the transition to floral development on the mainstem is associated with the prevention of further primary tiller buds being initiated. In the conditions of growth used in these experiments it appears that for a primary tiller to develop to maturity it must be initiated before the mainstem becomes floral in development, and must itself have produced 6 or more leaf initials before it receives the floral stimulus.

CHAPTER 4

EFFECTS OF MINERAL NUTRIENT APPLICATION ON EARLY TILLER BUD GROWTH IN BARLEY

As stated in the Introduction to this thesis (page 4) there are numerous reports that mineral availability has considerable effects on the extent of tillering in cereals; increased amounts of nitrogen in the soil medium are known to have a major effect in increasing the number of tillers emerging (Watson, 1936; Gregory, 1937), and non-nitrogenous minerals, including phosphorus and potas-:sium have also been shown to be important (Gregory, 1937). Aspinall (1961) found substantial effects of the timing, as well as of the quantity of the mineral supply, on tillering in barley. However there is an almost complete lack of data on the growth of tiller buds prior to their emergence from their subtending leaf sheaths (see page 17). The main purpose in the experimental work to be described in this chapter was, therefore, to inves-:tigate effects both of the timing and the extent of application of nitrogen and non-nitrogenous minerals on the early growth of tiller buds; it was hoped that these studies would contribute to an understanding of the mechanism and control of tillering.

I THE EFFECT OF DELAYING APPLICATION OF THE COMPLETE MINERAL NUTRIENT SOLUTION ON EARLY TILLER BUD DEVELOPMENT

The standard nutrient application to plants involved addition of both nitrate and non-nitrogenous minerals on day 4 after planting, and weekly subsequently; the quantities of each mineral supplied are given in chapter

2/

2 (page 24). Before studying the effects of components of the nutrient solution on early tiller bud growth, it was essential to investigate whether or not delay in the application of the complete mineral solution had obser-:vable effects on the initiation and early growth of tiller buds. The experiments to be described in this section were designed to investigate the effects of delay-:ing either the first or the second application of nutrient solution on tiller initiation, and early bud growth.

(i) The effect of delaying the initial application of nutrient solution on the total number of tillers initiated per plant by day 27

Standard quantities of the nutrient solution were applied initially on day 4, 8 or 11 and weekly subsequently, and an additional treatment received no supply of nutrient. For reasons outlined on page 92 day 27 was thought to be a suitable harvest date; on this date samples of six plants of each treatment were harvested, and the position of each tiller bud visible after dissection noted.

Numbers of primary, higher order, and total tiller buds visible on plants in the 4 treatments are shown in Table 4.1. Delay in the initial application of nutrient solution to day 8 did not significantly reduce the num-:bers of either primary or higher order tiller buds initiated, but further delay in nutrient application to day 11 had a considerable and significant effect, especi-:ally on the higher order tillers. In plants given no nutrient the effects on tiller initiation were even more severe. Treatments delaying initial application to day

8/

Table 4.1 Numbers of primary, higher order, and total tillers initiated per plant by day 27 after planting in control plants, and those having nutrient application delayed to day 8, 11, or never; 95% confidence limits are indicated.

Day of initial nutrient application	Tiller number		
	Primaries	Higher order	Total
4	4.5 ± 0.6	6.8 ± 1.8	11.3 ± 2.1
8	4.3 ± 0.5	5.0 ± 1.2	9.3 ± 1.4
11	3.5 ± 0.6	1.5 ± 1.1	5.0 ± 1.2
Never	3.3 ± 0.5	0	3.3 ± 0.5

8 or 11, and withholding nutrient completely reduced the total numbers of tillers initiated per plant by 18, 56 and 71% respectively compared to the value in the control treatment.

It is therefore clear that delay in nutrient application had a very marked effect on tiller bud initiation, especially that of the higher order tillers. No measurements of the size of the whole plants in the conditions of delayed nutrient application were obtained, but there was certainly a substantial effect on the growth of the whole plant as well as of the tillers.

(ii) The effect of delaying the initial application of nutrient solution on early growth of tiller buds TC and T1

To investigate the effects of delaying the application of the complete mineral nutrient solution on rates of plant and tiller growth, plants were supplied with the standard nutrient solution initially on either day 2, 4, 6, 8, 10 or 12, and weekly subsequently. Samples of 10 plants were harvested at 2, 3 or 4 daily intervals, and the dry weights of the tiller buds measured. Whole plant dry weights were also obtained in the treatments given nutrient initially on day 6, 8, 10 or 12. The graphs (Fig. 4.1 A,B) show clearly that the tillers TC and T1 increased in dry weight exponentially from the time of nutrient application to the end of the experiment, and it was therefore possible to determine the lines of best fit using the data obtained from the harvest immediately prior to nutrient application and all subsequent values; the regressions are shown in Fig./

Figure 4.1 The effects of delaying application of nutrient solution from day 2 to day 12 on growth in dry weight (μg) of tillers TC (A, C) and T1 (B, D). Raw data are shown in A and B, and linear regressions on these data in C and D.

Fig 4.1

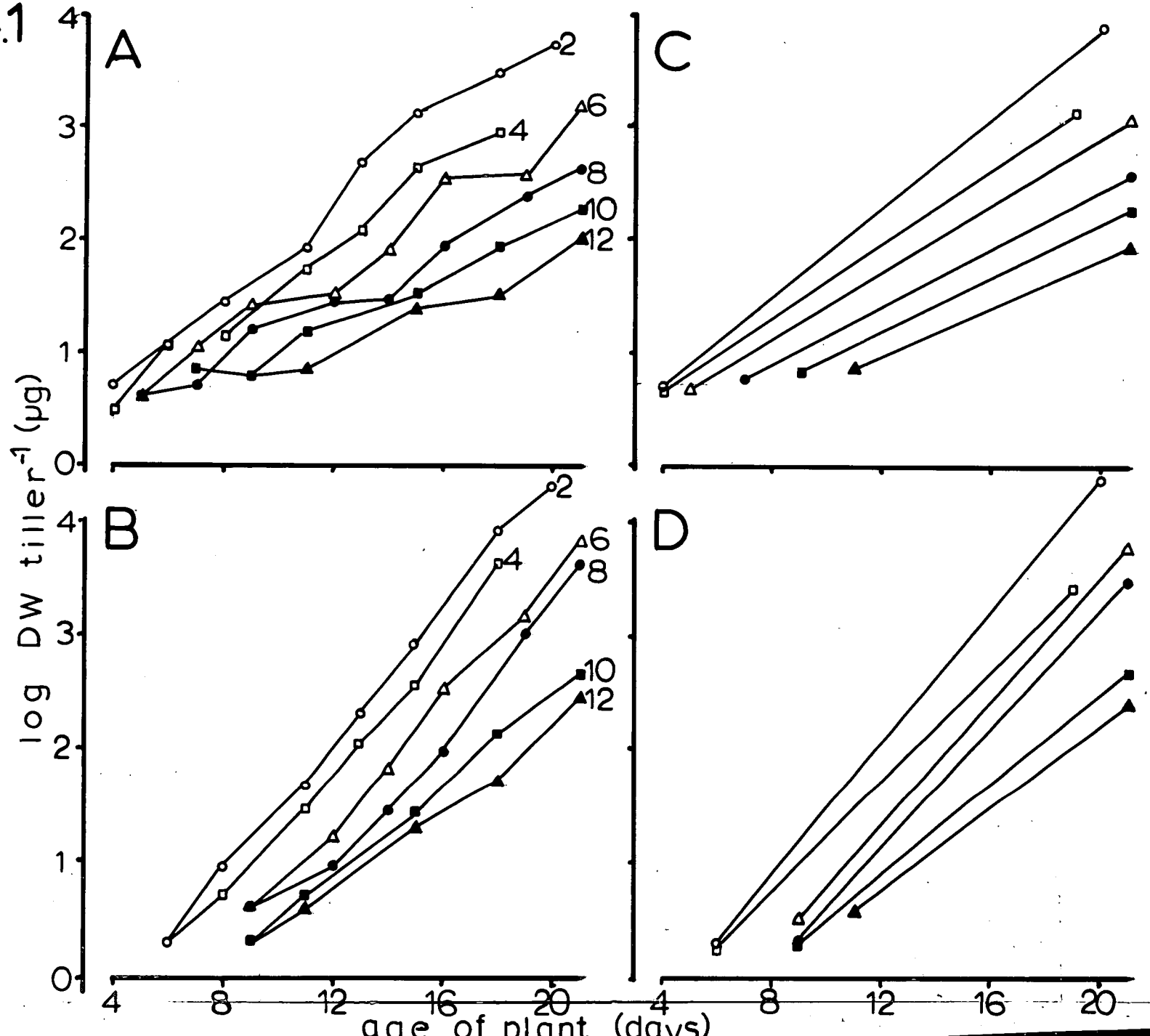

A
B
C
D
2
4
6
8
10
12
log DW tiller^{-1} (µg)
0
1
2
3
4
4
8
12
16
20
age of plant (days)

Fig. 4.1 C,D. In the case of the day 4 nutrient application the results from two runs were used in calculating the linear regression. Correlation coefficients for all the linear regressions were greater than 0.98, indicating that over 96% of the variation in each set of data was accounted for by the regression. Before the initial application of nutrient, growth of the coleoptile tiller TC was very slight, and its dry weight remained more or less constant at 4 - 7 µg. There was an immediate response to nutrient application and exponential growth continued to the end of the experiment. The weight of T1 increased slightly prior to nutrient application, and subsequently more rapidly; the regression coefficients were always higher for T1 than for TC in plants having the same nutrient treatment (Table 4.2), and by the end of the experiment T1 was always larger than TC.

The regression coefficient, a measure of the rate of exponential growth for the control, day 4, treatment was compared with that of each of the other treatments. As nutrient application was delayed the regression coefficients for TC decreased, and the values for day 8, 10 and 12 applications were all significantly lower than that for the day 4 application ($p = 0.05$, 0.01 and 0.01 respectively). The regression coefficient for the day 2 application was significantly higher than the control value ($p = 0.01$). When regression coefficients of T1 were compared with the day 4 application value it was found that, although values for day 2, 6 and 8 applications were higher than that for the control, only the day/

Table 4.2 The effect of delay in nutrient application on mean Relative Growth Rates ($gg^{-1}day^{-1}$) of tillers, TC and T1, and the whole plant. Period (days) over which R was measured is shown in parentheses.

Day of nutrient application	TC	T1	Whole Plant
2	0.46 (4-20)	0.69 (6-20)	-
4	0.38 (4-19)	0.56 (6-19)	-
6	0.35 (5-21)	0.63 (9-21)	0.15 (9-21)
8	0.33 (7-21)	0.60 (9-21)	0.13 (9-21)
10	0.27 (9-21)	0.46 (9-21)	0.10 (9-21)
12	0.25 (11-21)	0.42 (11-21)	0.11 (11-21)

day 2 result was statistically significant ($p = 0.05$). Values for T1 in the day 10 and 12 applications were lower than the control value, but these differences were not significant at the 5% level.

It is clear that TC was affected to a greater extent than T1 by the delay in application of mineral nutrient, since although the regression coefficients of both tillers decreased as nutrient application was further delayed, the differences were more marked in the case of TC than of T1*

Growth of the whole plant was also affected by treatment involving delay in application of nutrient solution, and on day 21 plants having nutrient supplied on day 6 were twice as heavy as those supplied with nutrient on day 12. Relative growth rates of tillers and plant were calculated, and the values for the tillers were always found to be higher than those for the plant in corresponding treatments (Table 4.2). When reductions in relative growth rates resulting from delayed application of nutrients were calculated as percentages (Table 4.3) there was no evidence of a different effect of the treatment on growth of tillers compared with that of the whole plant.

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* The results given above differ slightly from those given in Fletcher and Dale (1974), although the original data used in the calculations were the same in both cases. This is because for the paper the linear regressions of the growth curves were calculated using the $\log_{10}$ value of the arithmetic mean of the tiller bud dry weights for each sample, whereas in this thesis the mean of the $\log_{10}$ values has been used for each set of data. The two sets of results therefore differ in some details, but the overall conclusions are the same in both cases.

Table 4.3 Percentage reductions in the values of relative growth rates for tillers TC, T1 and whole plant, as the day of application of the complete mineral nutrient solution was delayed from day 6 to day 12.

			TC	T1	Plant
Day 8	cf.	Day 6	6	5	13
10	"	6	23	27	33
12	"	6	29	33	27
10	"	8	18	23	23
12	"	8	24	30	15
12	"	10	7	9	-

It can be concluded from this experiment that delay-:ing the initial application of the complete mineral nutrient solution delays the start of rapid increase in dry weight in tillers, and also reduces the rate of expo-:nential increase in dry weight in tillers and plant. The effects of delayed nutrient application are more marked in the case of TC than that of T1, but there is no evidence of a relatively greater effect on the tillers than on the plant, or vice versa.

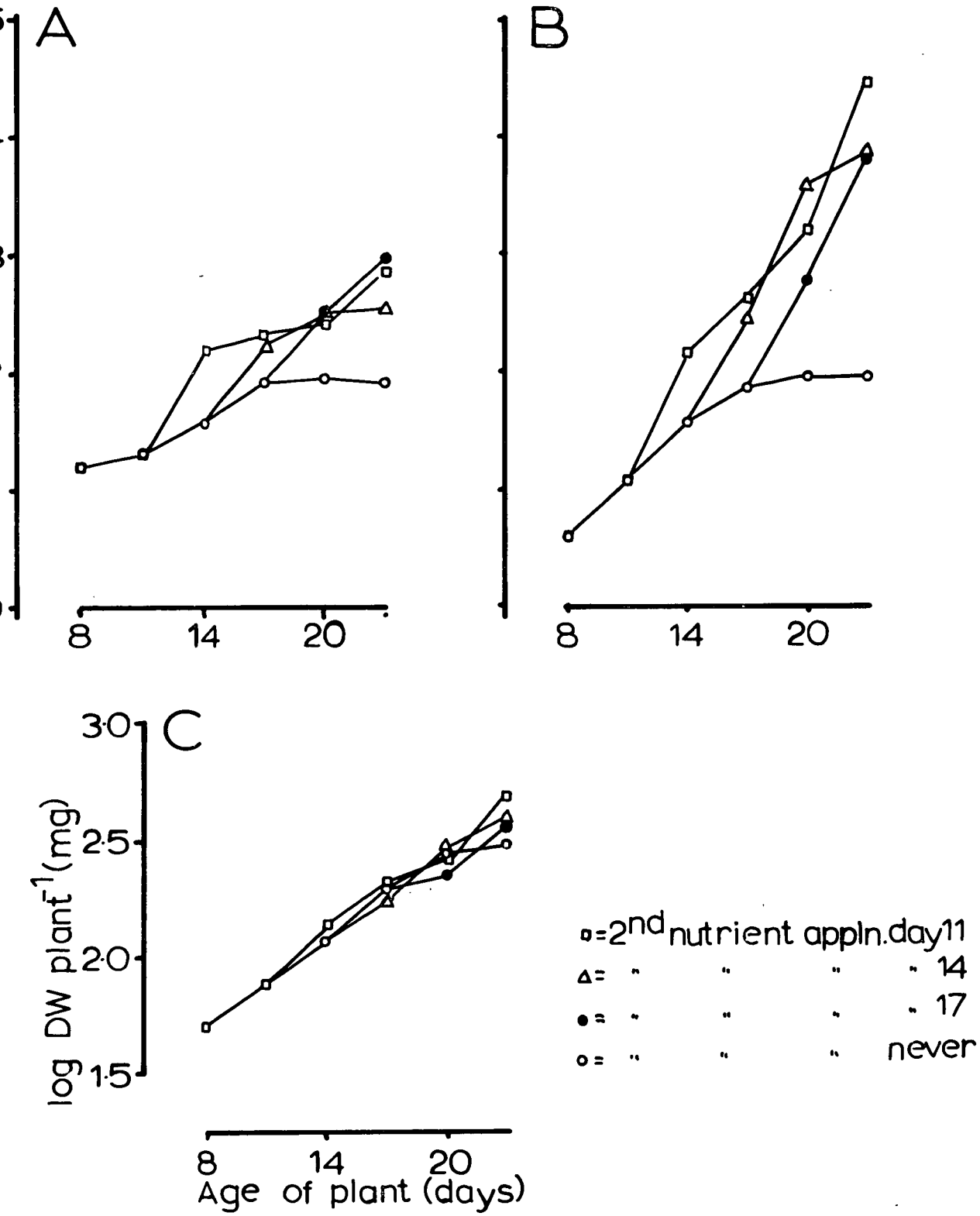
(iii) The effect of delaying the second application of nutrient solution on the growth of tiller buds TC and T1, and the whole plant

Having shown a considerable effect on tiller and plant development through delaying the initial application of nutrient solution it was of interest to investigate the effect of delaying the second application. All plants were supplied with nutrient on day 4 after planting and a second application of the standard quantities of minerals was made either on day 11, 14 or 17, or never. Samples of 9 plants from each treatment were harvested every third day from day 11 to day 23, and results for TC, T1 and the plant are shown in Fig. 4.2 A, B and C respectively.

Dry weights of the plant increased at each successive harvest up to day 23 in all the treatments (Fig. 4.2 C). However, on a particular harvest day the dry weights of plants in the four treatments did not necessarily de-:crease in the order in which plants had received the second application of nutrient; also, plants receiving the second application either on day 11 or never did not necessarily/

Figure 4.2 The effects of making the second application of nutrient solution on day 11, 14, 17 or never, on growth in dry weight of tillers (μg) TC (A) and T1 (B), and the plant (mg) (C).

g 4.2


A
B
C
8
14
20
3·0
2·5
2·0
1·5
log DW plant⁻¹(mg)
Age of plant (days)
□=2nd nutrient appln. day 11
△= " " " " 14
●= " " " " 17
○= " " " never

necessarily have the highest and lowest dry weights respectively. Using the 95% confidence limits to compare dry weights of the plant in the treatments in which nutrient was supplied on day 11 or never (Table 4.4), a significant difference was found for the harvest on day 14; the results for day 17 and 20 harvests were not significant, so that it appears that the day 14 result is of no biological importance. The result for the day 23 harvest was significant, and it seems probable that the plant was adversely affected by this date as a result of withholding the second application of nutrient; results from a further harvest date would have been required to confirm this suggestion.

Dry weights of the tillers in the two control treatments having nutrient supplied on day 11 or never, are now examined. Tiller dry weights did not increase at each harvest date in the treatment having no second application of nutrient, and from day 17 onwards both TC and T1 showed negligible increases in dry weight (Fig. 4.2 A, B); on day 23 each of these tillers weighed approximately 100 µg, whereas in the plants supplied with nutrient on day 11 the weights of TC and T1 were approximately 10 and 100 times greater respectively. On each harvest day tillers at each node having the lowest dry weights were found in the treatment in which no second application of nutrient was made. Using the 95% confidence limits to make comparisons between the treatments in the harvests from day 14 onwards (Table 4.4), it was found that of 8 comparisons, differences in dry weight reached a significant level/

Table 4.4 Log dry weights of plant (mg) and tillers (µg) TC and Tl on harvest days 14, 17, 20 and 23 in plants either supplied with a second application of full nutrient solution on day 11, or given no second application. 95% confidence limits are indicated, and significant differences between treatments are shown by an asterisk.

Harvest Day	Day of Second Application of Nutrient	Plant		TC		Tl	
14	11	2.14 ± 0.025	*	2.19 ± 0.210	*	2.16 ± 0.157	*
	Never	2.07 ± 0.052		1.58 ± 0.073		1.23 ± 0.158	
17	11	2.32 ± 0.051		2.32 ± 0.560		2.64 ± 0.590	*
	Never	2.29 ± 0.028		1.92 ± 0.128		1.86 ± 0.142	
20	11	2.42 ± 0.078		2.38 ± 0.365	*	3.21 ± 0.546	*
	Never	2.43 ± 0.043		1.96 ± 0.183		1.95 ± 0.216	
23	11	2.69 ± 0.038	*	2.87 ± 0.210	*	4.42 ± 0.137	*
	Never	2.48 ± 0.069		1.92 ± 0.212		1.95 ± 0.265	

level in 7. It was therefore apparent that dry weight increase in the tiller buds was adversely affected from day 14 onwards when the second application of nutrient was withheld.

Examination of the dry weight data reveals several differences between tillers and plant in their responses to the second application of nutrient being withheld. There was a significant difference in dry weight from day 14 onwards in the tillers, but only on day 23 in the plant, and whereas tiller increase in dry weight ceased from about day 17 in plants having no second application of nutrient, increase in plant dry weight continued to the end of the experiment.

Delaying the second application of nutrient to either day 14 or 17 resulted in an inhibition in tiller growth, but both tillers responded to the second application within three days (Fig. 4.2 A, B). On day 23 the TC tiller in the treatment having no second nutrient application had a significantly lower dry weight than the day 14 treatment, which in turn had a significantly lower dry weight than either the day 11 or the day 17 treatments. Dry weights of TC tillers in the latter two treatments did not differ significantly from each other. In the case of T1, tiller dry weight in the treatment given no second nutrient application was significantly lower than that in either of the treatments involving application of nutrient on day 14 or 17. In the control, day 11 treatment the T1 tiller was significantly larger than that in any of the other treatments on day 23. Results for/

for T2 showed similar significant differences to those for T1 in the various treatments. There was a progressive decrease in the dry weights of whole plants on day 23 as the second application of nutrient solution was further delayed, and the significant effects between treatments were the same as those for the tiller T1.

These data show that for continued growth of the tillers, TC, T1 and T2, a second application of nutrient is necessary, and that a delay in the supply of nutrient beyond day 11 causes a delay in the increase in dry weight of these tillers. The effects on growth of the plant are less clear cut, although by day 23 significant differences in dry weights between the various treatments are similar for both the plant and the tillers. It can be concluded that tiller development is more severely inhibited than that of the whole plant as a result of delaying the second application of nutrient.

(iv) Discussion

Application of the complete mineral nutrient solution later than day 4 after planting caused a delay in the onset of exponential growth in both tillers and the plant; in the case at least of the tiller TC there was also a decrease in the rate of exponential growth as nutrient application was progressively further delayed. This result suggests that TC's potential for growth was reduced by the treatment, although T1 does not appear to have been affected to the same extent; this finding will be examined more fully in the discussion at the end of this chapter (page 204).

Growth/

Growth of the tillers was also studied in plants having the initial application of nutrient on the day of planting, although the results of this run are not included in Fig. 4.1 (page 125). It was found that neither start, nor rate of exponential growth in the tillers was affected when plants were supplied with nutrient on day 0 rather than day 2. The most probable reason for this is that the roots of the seedling do not start active uptake of minerals until about day 2 after planting, so that application of nutrient on day 0 is of no extra advantage.

Withholding the second application of nutrient had substantial effects on growth of the tiller buds, this result being more marked than had been expected since the amounts of minerals supplied on day 4 are substantial. Also, it is known that a large proportion of the nitrate ions taken up by the plant over the first week after the initial nutrient application remains in the first leaf and roots as free nitrate (Dale, Felippe and Marriott, 1974). In the discussion at the end of this chapter, it is argued that nitrogen is an element of particular importance in tiller growth; it had been thought that the free nitrate in the first leaf and the roots would have been available for continued tiller growth. However, the experimental results make it clear either that this supply of nitrate is insufficient, or else that it is some other element that is limiting tiller bud growth in the absence of the second application of nutrient.

Marked effects of mineral supply on both plant and tiller/

tiller growth were found in all the experiments described in this section; in one experiment, that in which the second application of nutrient was delayed, the nutrient treatment was found to inhibit tiller growth to a greater extent than that of the plant. This result indicates that the pattern of plant growth is altered through mineral nutrient limitation, so that development of the axillary buds is suppressed in adverse conditions of mineral nutrition. Further evidence for this effect will be presented in this chapter, and a fuller discussion of apical dominance in barley is given in Chapter 6 (page 232).

Delay in the initial application of nutrient to day 11 after planting also had large effects on the numbers of tiller buds initiated, especially those developing from primary tillers. In the conditions of growth used the barley mainstem usually has 10 or 11 leaves, so that the number of positions from which primary tillers can develop is limited; substantial development of the pri-:mary tillers produces many more positions in which the higher order tillers can be initiated. Thus for plants to produce large numbers of tillers, initiation and de-:velopment of the secondary and tertiary buds is essential. With delay in initial nutrient application the initiation of primary tiller buds was little affected; however their early growth was limited, and this resulted in a reduced number of positions from which higher order tillers could develop. Thus the main effect of delayed nutrient application on initiation of tiller buds was on the higher order buds.

II/

II EFFECTS OF NITROGEN APPLICATION ON THE EARLY GROWTH OF THE PLANT AND TILLER BUDS

Having found a considerable effect on the early growth of tiller buds by delaying the application of the complete mineral nutrient solution it was of interest to investigate effects of components of the solution on the growth of tiller buds. Important effects of nitrogen content of the growth medium have already been mentioned (page 4); Dale (1972) showed a substantial effect of delay in nitrogen application on the early growth in dry weight of barley, although delay in application of mine-:rals other than nitrogen had a negligible effect. These results suggested that the main effects of delayed nutri-:ent application on tiller bud growth could be due to the delay in application of nitrogen, and experiments were designed to test this possibility.

Other experiments to be described in this section investigated effects on early plant and tiller growth of supplying nitrogen as ammonium rather than nitrate, and of varying the concentration of nitrate supplied.

(i) The effect of delaying the application of nitrogen

Two experiments were carried out to investigate the effects of delaying application of nitrogen to plants supplied with other minerals on day 4 after planting. Nitrogen, either as nitrate or ammonium, was supplied to the plants on day 4, 6, 8, 10 or 12 after planting; toxic effects of the ammonium ion on cereal growth are known (Jackson and Volk, 1967), and have been confirmed for barley under these conditions (Dale, Felippe and Marriott, 1974),/

1974), and therefore the study of plant and tiller growth was confined to a short 7 day period following nitrogen application. Samples of 8 plants were harvested 3 and 7 days after nitrogen application, and, in the control series not supplied with nitrogen, samples were harvested every second day from day 5 to 15, and day 7 to 17 in the ni-:trate and ammonium runs respectively.

There was little growth of TC in the absence of added nitrogen (Fig. 4.3 A, B), but substantial growth occurred within 3 days of the date of supply of nitrogen, although the response to ammonium was less marked than that to nitrate. In the case of T1, some growth occurred after about day 10, even in the absence of nitrogen (Fig. 4.3 C, D). This growth was greater than that found for TC in plants not supplied with nitrogen. More rapid growth of T1 occurred after the application of nitrogen, and the response to nitrate was again greater than that to ammonium. Changes in plant dry weight in response to added nitrogen (Fig. 4.3 E, F) were detectable, but less consistent than those of the tiller buds. Again response to ammonium nitrogen was less marked than that to nitrate.

Relative growth rates for both TC and T1 over a 4 day period from 3 days after nitrogen application (Table 4.5) were broadly similar for treatments involving the same nitrogen source, with rates for T1 always being greater than those for TC. As shown in the graphs (Fig. 4.3) the growth rates of buds supplied with ammonium were more variable than those in the nitrate series and generally both plants and tillers had greater growth rates/

Figure 4.3 The effect of delaying application of nitrogen as nitrate or as ammonium on growth in dry weight of tillers (μg) TC (A, B) and T1 (C, D) and the plant (mg) (E, F).

Fig 4.3

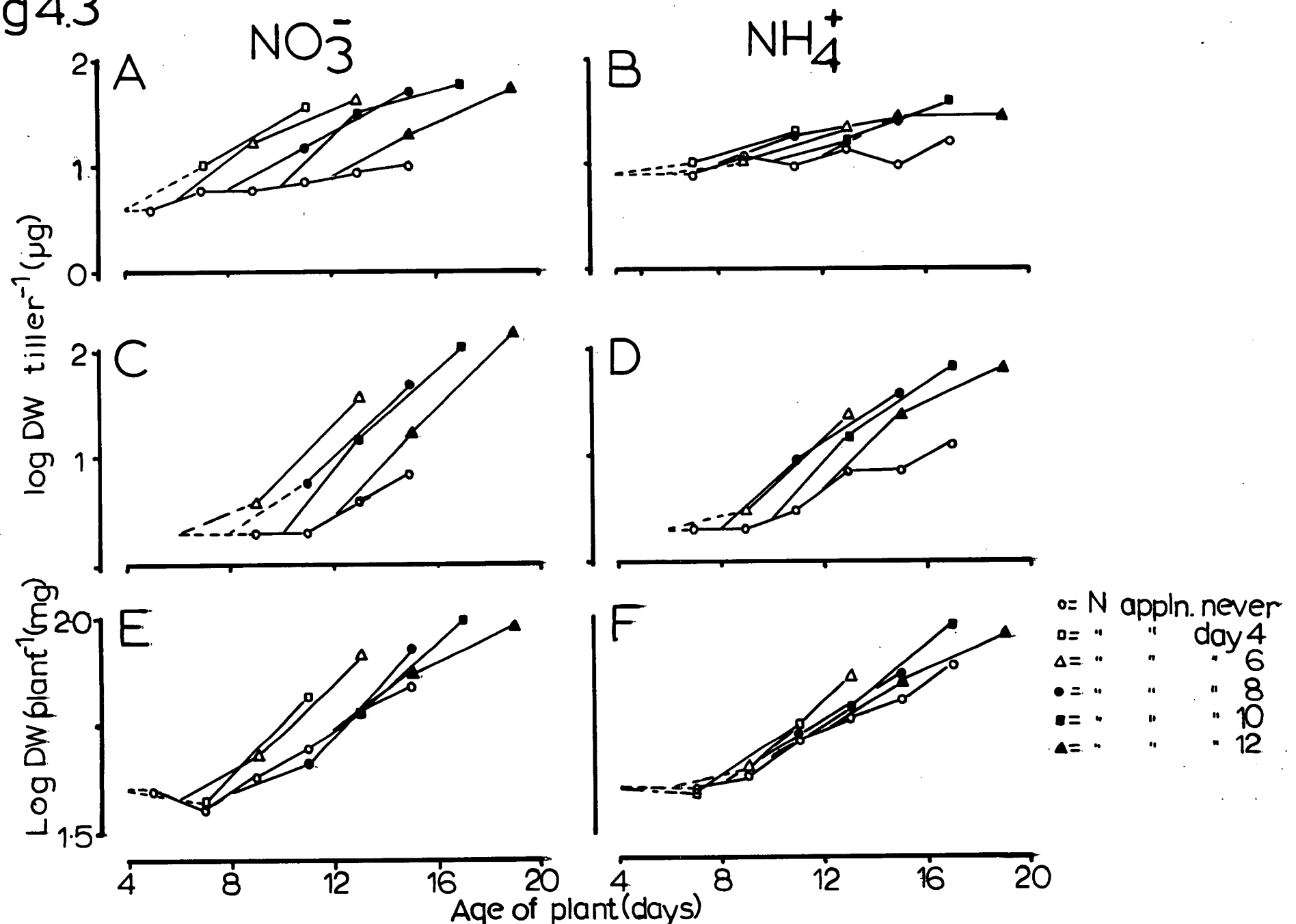

NO3-
NH4+
A
B
C
D
E
F
log DW tiller-1(µg)
Log DW plant-1(mg)
2
1
0
20
1·5
4
8
12
16
20
Age of plant (days)
o= N appln. never
□= " " day 4
Δ= " " " 6
●= " " " 8
■= " " " 10
▲= " " " 12

Table 4.5 Mean Relative Growth Rates (gg^{-1} day^{-1}) of tillers and whole plant over the period 3 - 7 days after application of nitrate. Results from an experiment in which ammonium was applied in place of nitrate are shown in parentheses.

Day of nitrogen application	Days over which R measured	TC	T1	Plant
4	7 - 11	0.32 (0.18)	- (0.43)	0.14 (0.09)
6	9 - 13	0.23 (0.17)	0.56 (0.52)	0.14 (0.13)
8	11 - 15	0.30 (0.08)	0.53 (0.36)	0.15 (0.08)
10	13 - 17	0.16 (0.22)	0.50 (0.38)	0.12 (0.11)
12	15 - 19	0.25 (0.02)	0.56 (0.26)	0.06 (0.06)
Never	7 - 15	0.06 (0.02)	- (-)	0.08 (0.06)
Never	9 - 15	- (-)	0.21 (0.21)	- (-)

rates when supplied with nitrate rather than ammonium. For the nitrate set the data do not indicate a clear-cut decrease in relative growth rates of the tillers or the plant with successive times of nitrogen application. This suggests that there was no decrease in the tillers' potential for growth through delay in nitrate application to day 12; whereas delay in the application of the full nutrient solution caused a progressive decrease in the relative growth rate of the TC tiller (see Table 4.2, page 127).

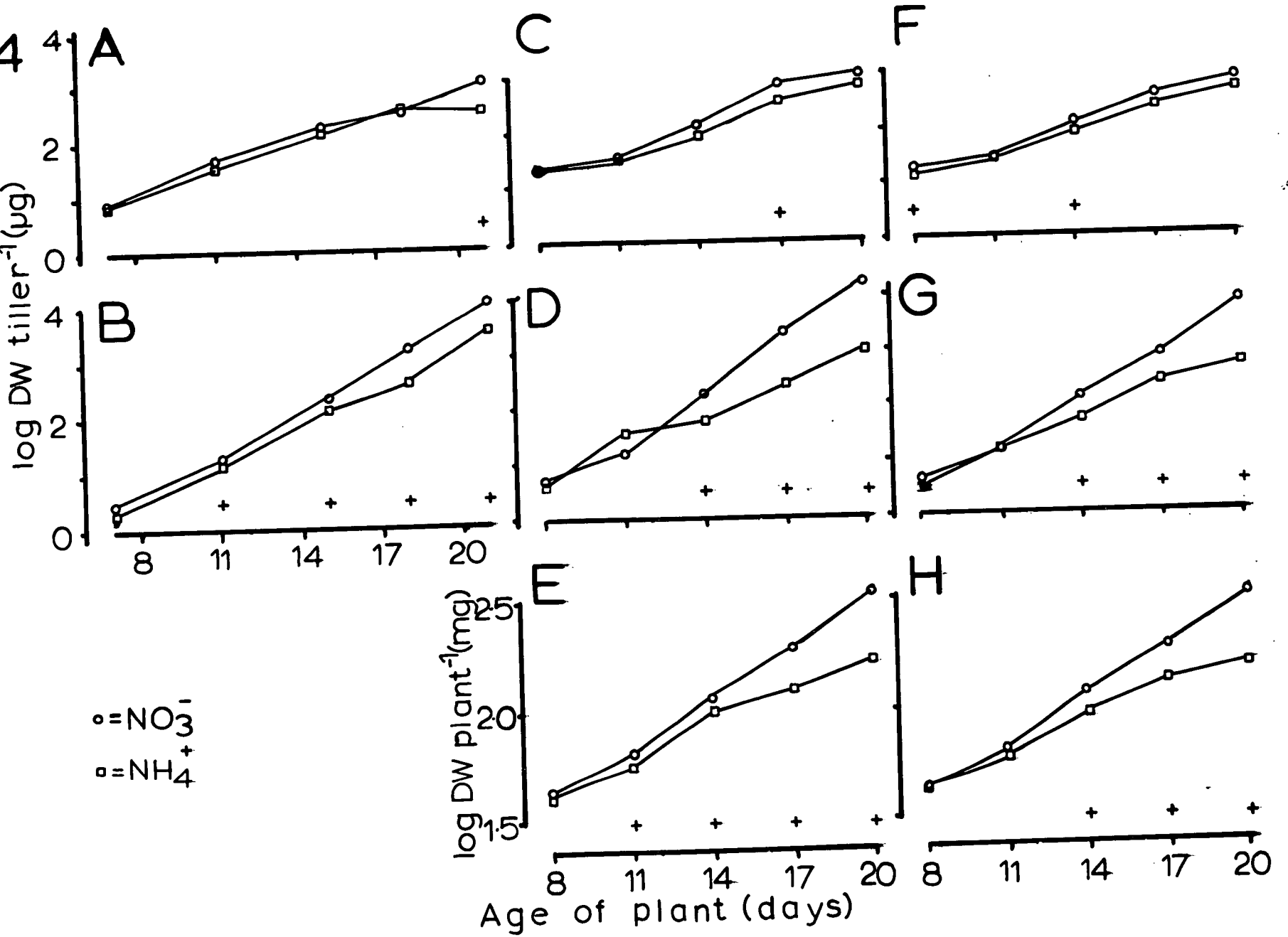
For the ammonium set the data are more variable, as already stated, and how far any decline in growth rate with delayed nitrogen application may be attributable to toxicity effects or to other factors, is not at all clear.

(ii) Comparison of plant and tiller bud growth in plants supplied with ammonium rather than nitrate

Results from the experiment just described suggested that growth of barley plants was promoted less by the supply of ammonium than by nitrate, and that this effect was noticeable within 7 days of nutrient application. To investigate the ammonium response further in plants up to three weeks old, two experiments were carried out in which nitrogen either as ammonium or as nitrate was added with the rest of the nutrient solution on day 4 after planting, and weekly subsequently. The results of both experiments are shown in Fig. 4.4. In the first experiments (Fig. 4.4 A, B) standard amounts of nitrogen were added (14mg pot^{-1} $application^{-1}$), and samples of 8 plants were harvested on days 7, 11, 15, 18 and 21. In the second experiment either 14mg (Fig. 4.4, C - E) or 7mg (Fig. 4.4, F - H) nitrogen,/

Figure 4.4 The effect of supplying nitrogen as ammonium or nitrate on growth in dry weight of tillers (μg) TC (A, C, F) and T1 (B, D, G) and the plant (μg) (E, H) in plants supplied with either full (A - E) or half (F - H) quantities of nitrogen. A - B and C - H refer to two separate experiments. An asterisk indicates a significant difference between samples based on 95% confidence limits.

Fig 4.4


A
B
C
D
E
F
G
H
log DW tiller^{-1}(µg)
log DW plant^{-1}(mg)
0
2
4
1·5
2·0
2·5
8
11
14
17
20
Age of plant (days)
○=NO_3^-
□=NH_4^+

nitrogen, either as ammonium or nitrate, were supplied to each plant on each application, and samples of 8 plants were harvested on days 8, 11, 14, 17 and 20.

When nitrogen was supplied at either 14 or 7 mg per application it was apparent that T1 was more affected than TC if the nitrogen was supplied as ammonium rather than nitrate. T1 tillers were significantly heavier in plants supplied with nitrate than in those supplied with ammonium in the last 3 harvests of both experiments (Fig. 4.4, B, D, G), whereas there were no consistent effects on TC (Fig. 4.4, A, C, F). In the second experiment clear effects of the form of nitrogen supply were found on plant growth (Fig. 4.4, E, H), with nitrate producing heavier plants than those supplied with ammonium. These effects were statistically significant from days 11 and 14 onwards when plants were supplied respectively with 14 or 7 mg nitrogen per application.

These results indicate an effect of the form of nitrogen supply on growth of both tillers and the plant; the effects reach a significant level on day 11 or 14 for the plant, approximately day 14 - 15 for T1, but are not significant for TC.

(iii) The effect of the concentration of nitrogen on plant and tiller bud growth.

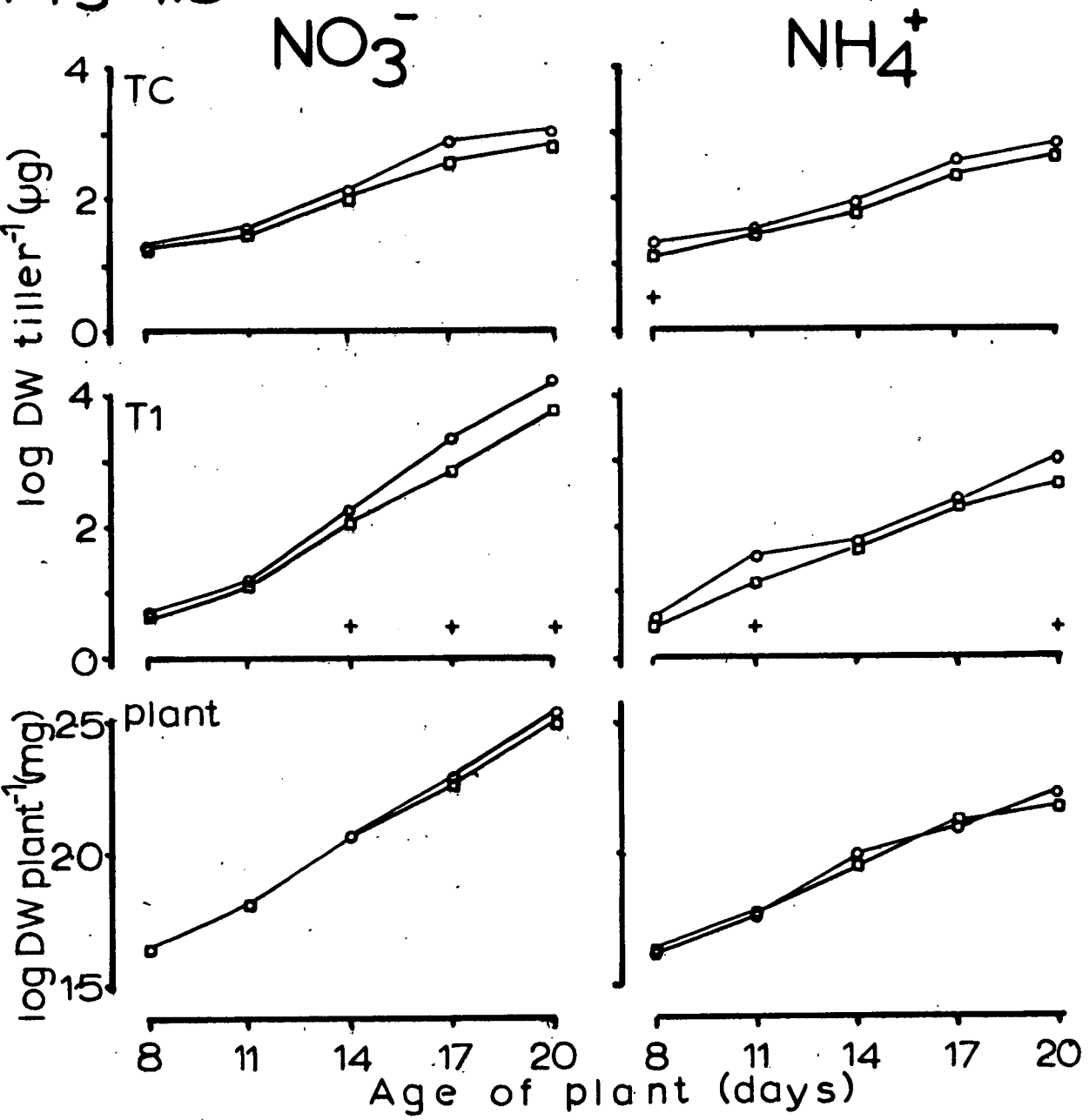
The experiment just described comparing growth of tillers and the whole plant when supplied with either nitrate or ammonium was also analysed to compare growth of plants supplied with either 14 or 7 mg nitrogen as either nitrate or ammonium. The results of this analysis are/

are shown in Fig. 4.5; dry weights of plants were not significantly different on any of the harvest days as a result of supplying 7 rather than 14 mg nitrogen. However, there were significant effects on the tillers; in plants supplied with 7 rather than 14 mg nitrogen, tiller T1 was significantly lighter on days 14, 17 and 20, in the nitrate treated plants, and on days 11 and 20 in plants supplied with ammonium. TC was significantly lighter in plants supplied with 7 rather than 14 mg nitrogen at the day 8 harvest, but since no significant effects were found on any of the other harvest days it seems probable that the day 8 effect was anomalous; in nitrate treated plants there was no effect on TC. These results indicated that growth of T1 was affected significantly by reduction of the amount of nitrogen supplied to 7 mg per application, whereas no effect was found on the growth of either the plant or TC. Other experiments were carried out to investigate further the effects of nitrogen concentration on early plant and tiller growth.

Two runs were carried out in which plants were supplied with varying amounts of nitrate, samples being dissected every 3 days from day 8 to day 20. In the first of these (Fig. 4.6, A - C) the sample size was 8 plants, and the control treatment, supplied with 14mg nitrogen per application, was compared on each harvest day with plants supplied with 2.8, 1.4 or 0.7 mg nitrogen per application. In the second run (Fig. 4.6, D - F) plants were supplied with 14, 7, 2.8 or 1.4 mg nitrogen per application, with 7 plants constituting each sample. Log dry/

Figure 4.5 The effect of supplying nitrogen at levels of either 14 or 7 mg per application on growth in dry weight of tillers (μg) TC and T1, and the plant (mg). Nitrogen was supplied either as nitrate or ammonium. An asterisk indicates a sig-:nificant difference between treatments based on 95% confidence limits.

Fig 4.5



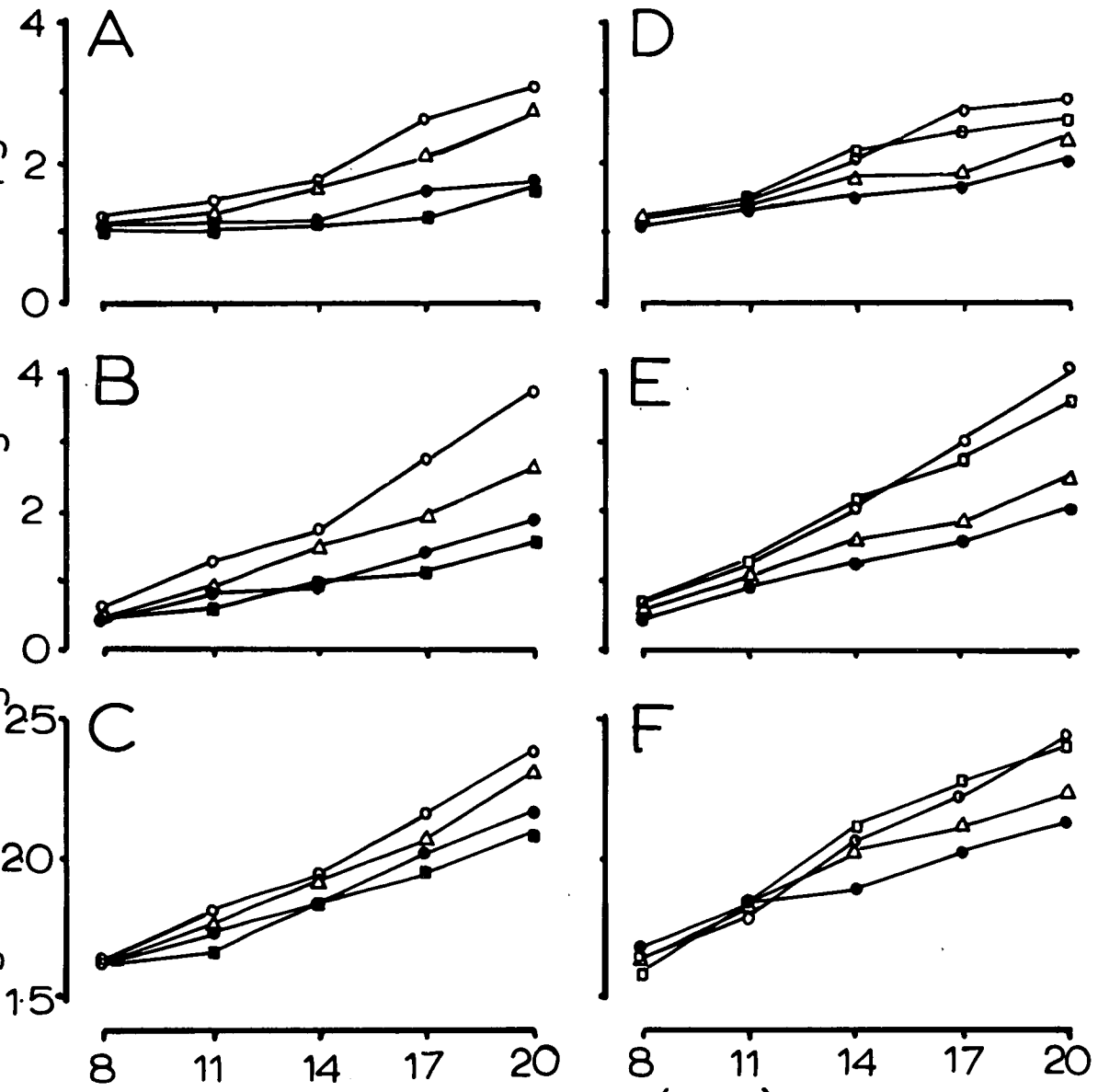
o=14mg N, □=7 mg N

Figure 4.6 The effect of supplying nitrate at 14, 7, 2.8, 1.4 or 0.7 mg nitrogen per application on the growth in dry weight up to day 20 of tillers (µg) TC (A, D) and T1 (B, E), and the plant (mg) (C, F). A - C, and D - F refer to two separate experiments. Significant differences, based on 95% confidence limits, in dry weights of tillers and plant between plants supplied with 14mg nitrogen per application, and those having lower amounts supplied were as follows. Results for the second experiment shown in D - F are given in parentheses.

* = significant difference;
ns = difference not significant;
o = comparison impossible

mg N supplied application^{-1}	Day of harvest	TC	T1	Plant
7	8	(o)	(o)	(ns)
	11	(ns)	(ns)	(ns)
	14	(ns)	(ns)	(ns)
	17	(ns)	(*)	(ns)
	20	(ns)	(*)	(ns)
2.8	8	o (ns)	o (o)	ns (ns)
	11	* (*)	o (o)	ns (ns)
	14	ns (*)	* (*)	ns (ns)
	17	* (*)	* (*)	ns (*)
	20	ns (*)	* (*)	* (*)
1.4	8	* (o)	o (o)	ns (ns)
	11	* (*)	o (o)	ns (ns)
	14	* (*)	o (*)	* (*)
	17	* (*)	* (*)	* (*)
	20	* (*)	* (*)	* (*)
0.7	8	*	o	ns
	11	*	o	*
	14	*	o	*
	17	*	*	*
	20	*	*	*

Fig 4.6



○ = 14 mg N application^{-1}
□ = 7 " " "
△ = 2·8 " " "
● = 1·4 " " "
■ = 0·7 " " "

dry weights of tillers TC and T1, and the whole plant were compared for the control treatment with those in treatments involving smaller supplies of nitrogen, and the significant effects are included in the legend to Fig. 4.6.

No significant effect on plant growth was found when 7 rather than 14 mg nitrogen were supplied, but application of only 2.8 mg nitrogen resulted in significant differences in plant dry weight compared with the controls on day 20 in the first experiment, and days 17 and 20 in the second. In both experiments the treatment in which 1.4mg nitrogen were supplied to plants resulted in a significant effect on plant dry weight being found from day 14 onwards, and in the ~~second~~ first experiment application of 0.7mg nitrogen per plant resulted in a significant effect on plant dry weight from day 11 onwards. Thus, as the amounts of nitrogen supplied to plants were reduced to 2.8, 1.4 and 0.7 mg per application, lower plant dry weights compared to those obtained in the controls were found at progressively earlier harvest dates.

No significant difference was found in the dry weight of TC up to day 20 when 7 rather than 14 mg nitrogen were supplied to each plant, but T1 was significantly lighter on days 17 and 20 in plants supplied with 7 mg nitrogen. Further reductions in the quantity of nitrogen supplied to each plant resulted in significant differences in tiller TC dry weight being found at progressively earlier harvest dates. The small size of the T1 tiller in plants supplied with low amounts of nitrogen meant that T1 buds could not always be weighed individually; groups of buds were/

were weighed together, and this meant that significant differences could not be calculated on the earlier harvest dates. Itwas therefore impossible to show a progressive effect of reduction in nitrogen supply on T1, as has been shown already for TC.

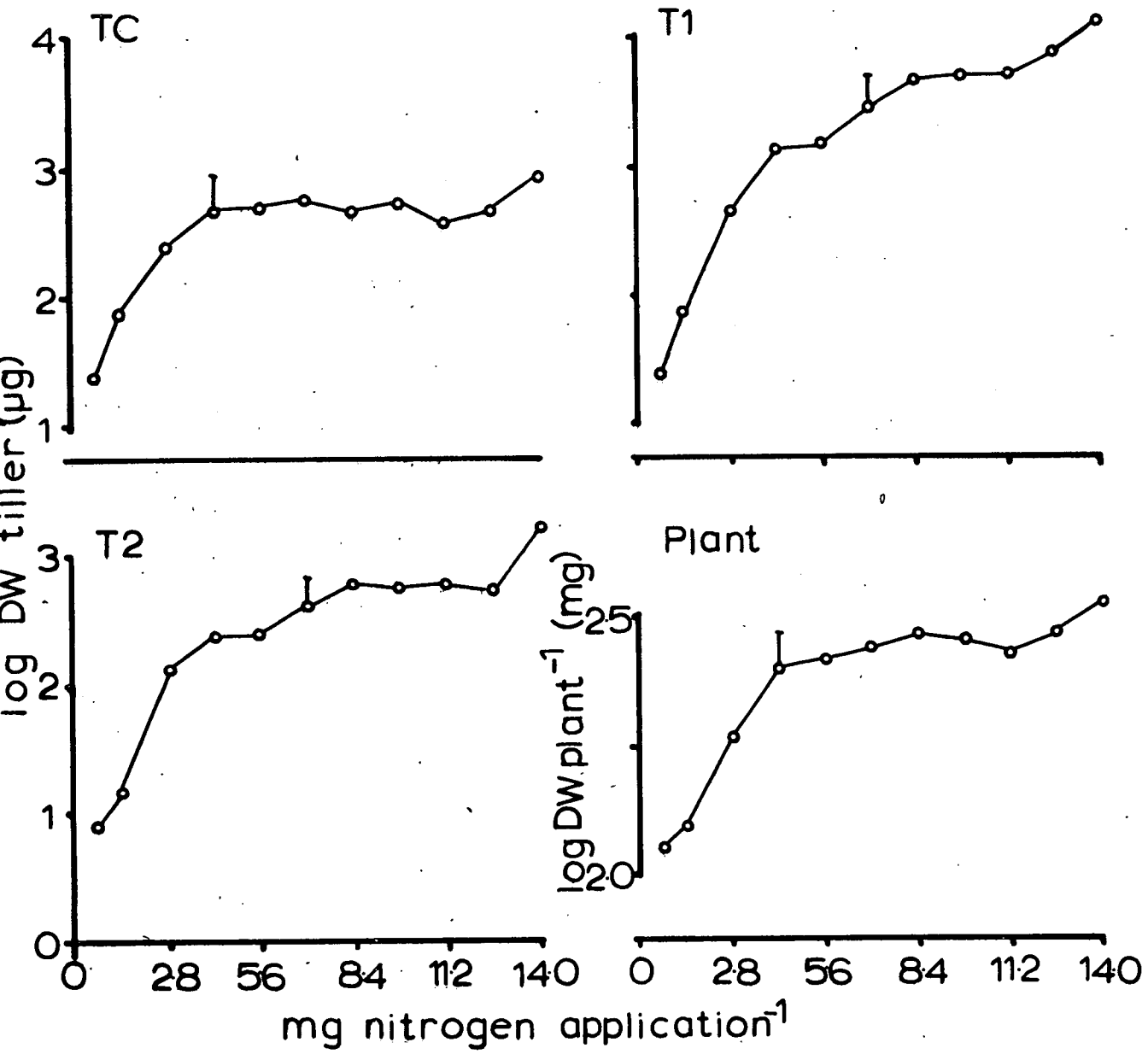
To summarise, significant effects were found at an earlier harvest date for tillers than the plant when nitrogen supply was reduced below the control level; tiller T1 was more affected than TC through reduction in the supply of nitrogen. Progressively greater effects on plant and tiller growth were found as the nitrogen supply was reduced still more.

In another experiment to investigate the effects of quantity of nitrogen supplied on the growth of tillers and plant, levels of nitrogen ranging from 14.0mg to 0.07 mg were supplied to plants of 11 treatments. Samples of 8 plants of each of the treatments were harvested on day 20 after planting, and the dry weights of tillers TC, T1 and T2, and the plant were obtained. Analyses of variance were carried out on the log data obtained for each struc-:ture, and the results are shown in Fig. 4.7. Values for TC and T2 in the plants supplied with 0.07 mg nitrogen could not be included in the analyses, since these tillers were too small to be weighed individually; however, means for these samples are included in Fig. 4.7.

Growth of the plant was not significantly increased when the nitrogen level was raised above 4.2mg per plant. However, for maximum growth of T1 and T2 a level of 7.0 mg nitrogen per plant per application was required; there was/

Figure 4.7 The effect of increasing nitrogen supply on dry weight of tillers (μg) TC, T1 and T2, and the plant (mg), harvested on day 20. Vertical bars indicate least significant differences ($p = 0.05$) computed from analyses of variance on the data.

Fig 4.7


TC
T1
T2
Plant
log DW tiller (µg)
log DW plant^{-1} (mg)
0
28
56
84
112
140
mg nitrogen application^{-1}

was therefore a difference between these tillers and the plant in their growth response to different levels of soil nitrogen, with T1 and T2 requiring a greater supply of nitrogen than the plant for maximum growth. Results for TC were rather more variable, due to the bimodality of the distribution of the tiller weights shown previously (page 104), but it appears that the maximum growth of TC was reached in plants supplied with 4.2 mg nitrogen; this was the same level as that required for maximum plant growth.

Results from plants supplied with 14.0mg nitrogen per application were significantly higher than the plateau values for T1, T2 and the plant, and in the case of T1 the value for plants supplied with 12.6mg nitrogen was also higher than that for the plateau. These results seem surprising in view of the fact that no significant difference in plant growth up to day 20 was found in the experiments just described (Fig. 4.5, 4.6; pages 150 and 152), in which applications of 7.0mg nitrogen were compared with control levels; it is probable therefore that the results for the 14.0mg nitrogen treatment were higher than the plateau values due to chance sampling of larger plants.

(iv) Summary

The experiments carried out to investigate the effects of nitrogen supply on plant and tiller growth confirm a major effect of nitrogen nutrition on plant development, with delay in supply of nitrogen, use of ammonium rather than nitrate, and reduction in the amount of/

of nitrogen supplied all having significant effects on plant and tiller growth. Taking the results from these experiments together it can be concluded that low levels of nitrogen application are more harmful with respect to tiller growth than to that of the whole plant, over the first three weeks of plant development. TC appears to be less affected than T1 in plants supplied with less than control amounts of nitrogen, and when ammonium rather than nitrate is used as the nitrogen source. All these results will be discussed more extensively later (page 195).

III EFFECTS OF DELAYING THE APPLICATION OF NON-NITROGENOUS MINERALS ON EARLY PLANT AND TILLER BUD GROWTH

An experiment has been described previously (page 139) in which non-nitrogenous minerals were supplied to the plant on day 4, with nitrogen being added some days later. This experiment showed that little growth of the tillers occurred until nitrogen, as well as non-nitrogenous mine-:rals had been supplied to the plant. It was now necessary to carry out the complementary experiment in which nitrogen was supplied to plants on day 4, and non-nitrogenous mine-:rals subsequently.

In the first of these experiments nitrogen, as ni-:trate, was supplied on day 4 after planting, and non-nitro-:genous minerals, at either full or $\frac{1}{5}$ amounts, on day 5, 8 or 11. Samples of 9 plants were harvested 3 and 6 days after application of non-nitrogenous minerals, and a con-:trol set, supplied only with nitrogen on day 4, was har-:vested every third day from day 5 to 17.

Dry weights of the plant increased throughout the experiment,/

experiment, even in the absence of a supply of non-nitro-:genous minerals (Fig. 4.8, E, F), and while slight increases in weight followed application of non-nitroge-:nous minerals these were significant only for the day 5 treatment in plants supplied with the control amount of non-nitrogenous nutrient. It might be expected that greatest effects would be shown when these nutrients were supplied late, and since this was not the case the bio-:logical significance of these small increases must be in doubt.

However, growth of both the tillers TC (Fig. 4.8, A, B) and T1 (Fig. 4.8, C, D) was affected by the supply of control amounts of non-nitrogenous minerals, both tillers being significantly heavier than those in plants having no application of non-nitrogenous minerals. Tiller T1 was significantly heavier in plants supplied with $\frac{1}{5}$ amounts of non-nitrogenous minerals on day 5 or 8, but results from the day 11 treated plants were vari-:able, the sample on day 14 being significantly lighter than the controls. Supply of $\frac{1}{5}$ amounts of non-nitrogenous nutrients did not significantly increase growth of the TC tiller bud.

Thus, delaying the application of non-nitrogenous minerals to plants already supplied with nitrogen showed no clear effect on plant growth, but significantly affected growth of both the buds TC and T1.

Relative growth rates of the tillers over the six day period after application of the control amounts of non-nitrogenous minerals (Table 4.6) were similar to those/

Figure 4.8 The effect of delaying the application of either full (A, C, E) or 1/5 (B, D, F) amounts of non-nitrogenous minerals on growth in dry weight of tillers (μg) TC (A, B) and T1 (C, D), and the plant (mg) (E, F). Significant differences, based on 95% confidence limits, in dry weights of tillers and plant between plants supplied with full amounts of non-nitrogenous minerals, and those not supplied were as follows. Results for application of 1/5 amounts of non-nitrogenous minerals are shown in parentheses.

* = significant difference;
ns = difference not significant;
o = comparison impossible;
\- = significant difference, but with control structure heavier than that in treatment with non-nitrogenous nutrient.

Day of nutrient application	Day of harvest	TC	T1	Plant
5	8	* (ns)	o (o)	ns (ns)
	11	* (ns)	* (*)	* (ns)
8	11	* (ns)	* (*)	ns (ns)
	14	* (ns)	* (*)	ns (ns)
11	14	* (ns)	* (-)	ns (ns)
	17	* (ns)	* (ns)	ns (-)

Fig 4.8

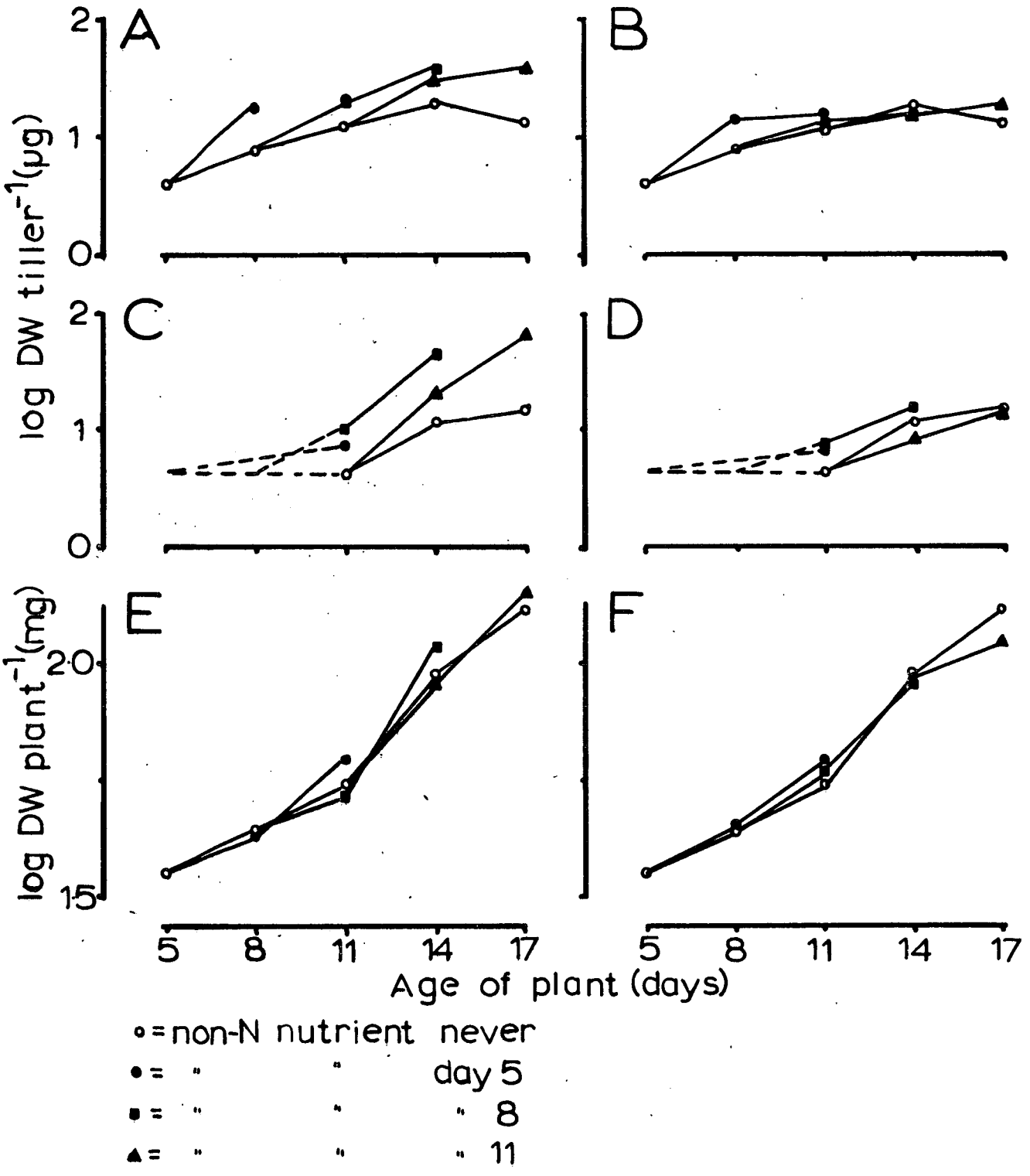

A
B
C
D
E
F
2
1
0
2
1
0
2.0
1.5
log DW tiller^{-1}(µg)
log DW plant^{-1}(mg)
5
8
11
14
17
Age of plant (days)
o = non-N nutrient never
● = " " day 5
■ = " " " 8
▲ = " " " 11

Table 4.6 Mean relative growth rates (gg^{-1} day^{-1}) of tillers and plant after application of non-:nitrogenous minerals on days 5, 8 or 11 to plants already supplied with nitrate. Results for set having 1/5 strength non-nitrogenous minerals are shown in parentheses.

Day of nutrient application	Days over which R measured	TC	T1	Plant
5	5-11	0.26 (0.23)	— —	0.10 (0.09)
8	8-14	0.27 (0.13)	— —	0.15 (0.14)
11	11-17	0.19 (0.07)	0.46 (0.20)	0.16 (0.12)
Never	5-17	0.10	—	0.11
Never	11-17	—	0.22	—

those found after nitrate had been supplied to plants previously given only non-nitrogenous minerals (Table 4.5 page 143), but in plants supplied with only 1/5 amounts of non-nitrogenous minerals relative growth rates were rather smaller (Table 4.6).

A comparison of growth in dry weight in plants having been supplied with either control or 1/5 amounts of non-nitrogenous minerals was also carried out. The data (Table 4.7) showed that lowering the amount of non-:nitrogenous minerals supplied to plants, but keeping nitrogen supply constant, had different effects on plant and tiller bud growth. Of 11 comparisons of tiller dry weight between the two treatments, 9 showed significant effects, with tillers heavier in plants supplied with control than with 1/5 amounts of non-nitrogenous nutrients. For the plant only 2 out of 6 comparisons between the treatments showed significant differences.

Comparison of Figs. 4.8 (page 161) and 4.3 (page 142) indicates that growth of both TC and T1 was rather higher when nitrogen was present without any non-nitrogenous minerals than in the complementary situation. However, this can only be a tentative conclusion, since the diffe-:rences could reflect slight differences in growth condi-:tions other than nutrient treatment in the two experiments.

The experiment just described was done on a relatively small scale, and therefore a second one similar, but not identical in design, was also carried out. In addition to investigating further the effects of delaying the application of non-nitrogenous nutrients this experiment studied/

Table 4.7 Log dry weights of tillers (μg) TC and T1 and the whole plant (mg) in plants supplied with either full or 1/5 amounts of non-nitrogenous minerals, and harvested 3 and 6 days after application of minerals on day 5, 8, or 11. 95% confidence limits are indicated. Significant differences between treatments are shown by asterisks.

Day minerals supplied	Harvest Day	Amount of non-nitrogenous minerals supplied	TC		T1		Plant	
5	8	Full	1.27 ± 0.039	*	-		1.64 ± 0.051	
		1/5	1.15 ± 0.050		-		1.65 ± 0.039	
	11	Full	1.28 ± 0.076		0.86 ± 0.082		1.80 ± 0.030	
		1/5	1.20 ± 0.121		0.82 ± 0.095		1.79 ± 0.052	
8	11	Full	1.29 ± 0.084	*	1.04 ± 0.099	*	1.72 ± 0.037	
		1/5	1.13 ± 0.061		0.88 ± 0.155		1.77 ± 0.052	
	14	Full	1.60 ± 0.289	*	1.66 ± 0.245	*	2.04 ± 0.051	*
		1/5	1.23 ± 0.159		1.18 ± 0.064		1.96 ± 0.029	
11	14	Full	1.49 ± 0.131	*	1.33 ± 0.142	*	1.96 ± 0.066	
		1/5	1.21 ± 0.059		0.93 ± 0.078		1.97 ± 0.051	
	17	Full	1.58 ± 0.189	*	1.82 ± 0.336	*	2.15 ± 0.031	*
		1/5	1.27 ± 0.240		1.15 ± 0.211		2.04 ± 0.035	

studied also the effect of supplying nitrogen as potassium rather than as sodium nitrate; potassium has been shown to be important in tiller development (Gregory, 1937), and it was therefore of interest to investigate whether or not the presence of potassium together with nitrogen allowed better growth of tiller buds than sodium with nitrogen. In this experiment plants were supplied with either potassium or sodium nitrate on day 4, and non-:nitrogenous minerals were added subsequently on either day 5 or day 11. Samples of 8 plants were harvested 3 and 6 days after application of the non-nitrogenous nutri-:ents, and the results are shown in Fig. 4.9. Similar results to those of the previous experiment were obtained, and the growth rates of tillers were higher in plants supplied with non-nitrogenous minerals as well as nitrate than in those supplied only with nitrogen. It was again found that tillers were more greatly affected than the plant as a result of addition of non-nitrogenous nutrients.

The dry weights of tillers and plants in the two treatments supplied with either potassium or sodium nitrate, but with no other minerals are shown in Table 4.8. Tillers are significantly heavier in 2 out of 6 comparisons in plants supplied with potassium rather than sodium; no 95% confidence limits could be calculated for tiller T1 in the harvests on days 8 and 11, since on these days the buds were too small to weigh individually. The weight of the whole plant was significantly greater in the day 11 harvest when potassium was supplied rather than sodium. There are therefore slight differences in the/

Figure 4.9 The effect of delaying the application of full amounts of non-nitrogenous nutrient on growth in dry weight of tillers (μg) TC (A, B) and T1 (C, D), and the plant (mg) (E, F) in plants supplied with nitrate either as sodium nitrate (A, C, E) or as potassium nitrate (B, D, F). Significant differences, based on 95% confidence limits, in dry weights of tillers and plant between plants supplied with non-nitrogenous minerals, and those not supplied, were as follows. Results for plants supplied with potassium nitrate are shown in parentheses.

* = significant difference;
ns = difference not significant;
o = comparison impossible.

Day of nutrient application	Day of harvest	TC	T1	Plant
5	8	* (ns)	o (o)	* (ns)
	11	* (ns)	o (o)	* (ns)
11	14	ns (ns)	ns (*)	ns (ns)
	17	ns (ns)	* (ns)	ns (ns)

Fig 4.9

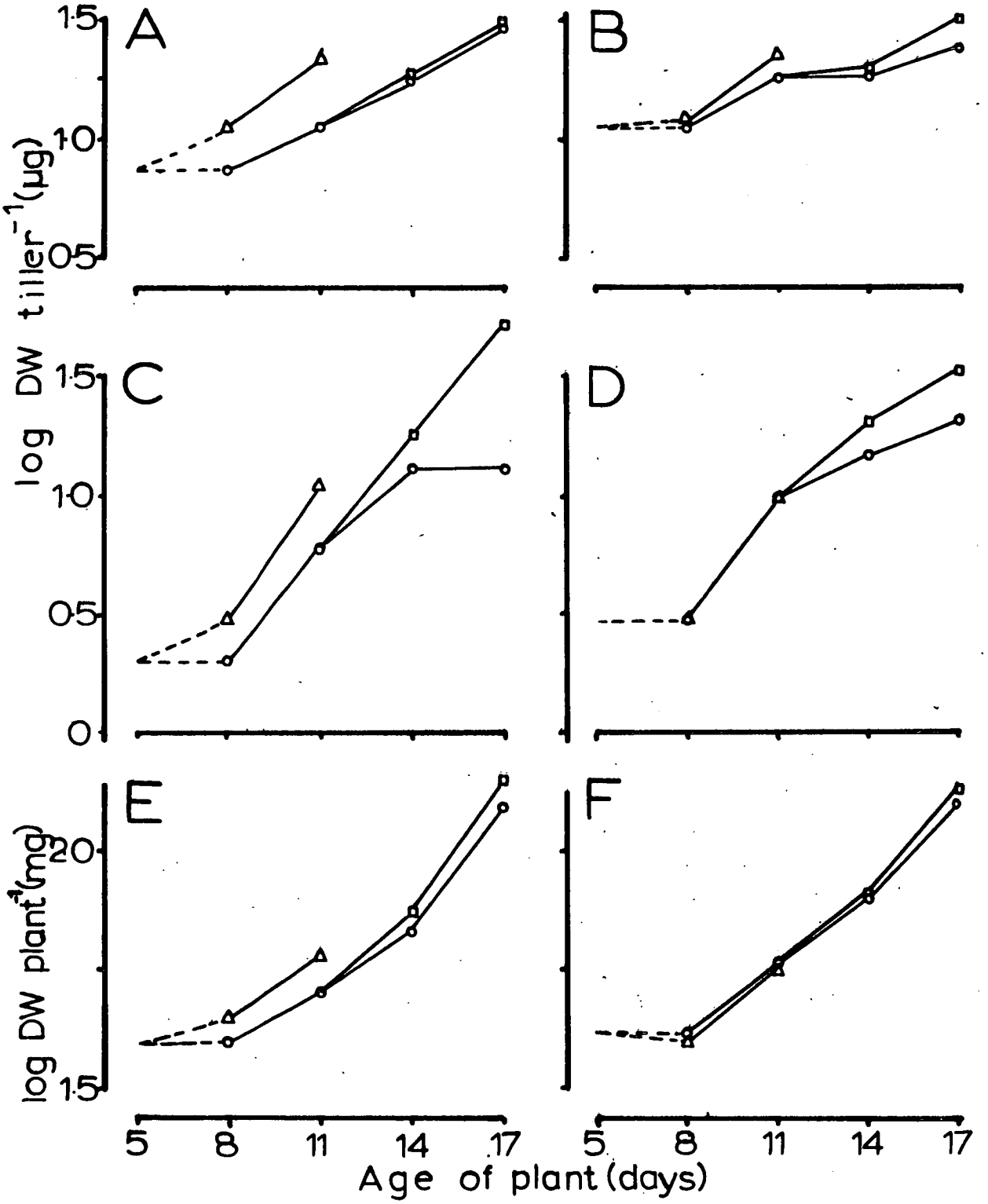

A
B
C
D
E
F
log DW tiller^{-1}(µg)
log DW plant^{-1}(mg)
1·5
1·0
0·5
0
2·0
1·5
5
8
11
14
17
Age of plant (days)
o = non-N nutrient never
Δ = " " day 5
□ = " " " 11

Table 4.8 Log dry weights of tillers (μg) TC and T1, and whole plant (mg), in plants supplied with nitrogen as either potassium or sodium nitrate, but having no supply of non-nitrogenous nutrients. 95% confidence limits are indicated, and significant differences are shown by asterisks.

Day of Harvest	Cation supplied with Nitrate	TC	T1	Plant
8	Na	0.87 ± 0.054 *	0.30	1.60 ± 0.033
	K	1.05 ± 0.142	0.48	1.62 ± 0.024
11	Na	1.05 ± 0.060 *	0.78	1.70 ± 0.040 *
	K	1.26 ± 0.207	1.00	1.77 ± 0.047
14	Na	1.25 ± 0.060	1.12 ± 0.025	1.87 ± 0.090
	K	1.27 ± 0.089	1.18 ± 0.067	1.90 ± 0.062
17	Na	1.47 ± 0.141	1.12 ± 0.040	2.10 ± 0.040
	K	1.39 ± 0.097	1.32 ± 0.234	2.10 ± 0.054

the dry weights of plants and tillers in plants supplied with potassium rather than sodium; however, these differences are not consistent enough to conclude that over the period investigated the supply of potassium allowed either more or less plant growth than that when sodium was supplied.

Thus, the evidence from both the experiments described shows that non-nitrogenous minerals are of great importance in the early development of tiller buds. Either delay in application, or reduction in the amount of these minerals supplied has a severe effect on growth of the tiller buds TC and T1, but an insignificant effect on growth in dry weight of the plant. No convincing increase in plant or tiller growth was evident when plants were supplied with potassium instead of sodium.

IV THE EFFECTS OF VARYING THE DATES OF APPLICATION OF BOTH NITRATE AND NON-NITROGENOUS MINERALS ON EARLY PLANT AND TILLER BUD GROWTH

In Section I of this chapter experiments were discussed in which application of the complete mineral nutrient solution was varied; in sections II and III data have been presented from experiments in which either nitrate or non-nitrogenous mineral application was varied. It was now of interest to investigate the effects on plant and tiller growth in dry weight of varying dates of application of both nitrogen and non-nitrogenous minerals. The experiments to be described in this section were therefore designed to extend the results already discussed, firstly by further examining the independent effects of delaying the application of either nitrogen/

nitrogen or non-nitrogenous nutrients, and secondly, by studying effects of both these variables on plant and tiller bud growth within the same experiment. It was hoped that using this approach an evaluation of the rela-:tive importance of the two parts of the mineral nutrient solution on early growth of plant and tiller buds would be possible. In all the experiments to be described in this section standard amounts of the nitrate and non-:nitrogenous nutrients were supplied to plants.

(i) A factorial experiment to investigate effects of, and interactions between time of harvest, and dates of application of nitrate and of non-nitrogenous nutrients

In this experiment nitrate was supplied on either day 4 or day 8 after planting. Non-nitrogenous minerals were applied on day 0, 4, or never, and samples of 6 plants of each of the 6 treatments were harvested on days 8, 12, 16 and 20. Further applications of the nutrient solutions were made 8 and 16 days after the initial treatments. This was a factorial experiment, and an analysis of vari-:ance was carried out on the log dry weight data of the tillers TC and T1 and the plant.

From Table 4.9 it is seen that there were a number of interactions and these will be discussed subsequently. Because of these it was not possible immediately to assess significance of the main effects; least significant differences ($p = 0.05$) were therefore calculated for these, and are given with the dry weight data in Table 4.10. At each successive harvest log dry weight clearly increases significantly for both the tillers and the plant (Table/

Table 4.9 Summary of interactions found in factorial experiment in which nitrate was supplied on either day 4 or 8, non-nitrogenous minerals on day 0, 4 or never, and plants were harvested on days 8, 12, 16 and 20.

Significant effects at $p = 0.05$(*) and $p = 0.01$(**) are shown.

Interactions	TC	T1	Plant
Nitrate x Time of harvest			**
Non-nitrogenous nutrient x Time of harvest	*	*	
Nitrate x non-nitrogenous nutrient	*	*	

Table 4.10 Log dry weights of tillers (μg) TC and T1, and the plant (mg) to indicate the main effects in the factorial experiment in which nitrate was supplied on either day 4 or 8, non-nitrogenous mine-:rals on day 0, 4 or never and plants were harvested on days 8, 12, 16 and 20.

± figures indicate least significant differences (p = 0.05)

		TC	T1	Plant
A. Day of harvest	8	0.94		1.58
	12	1.39	1.06	1.78
	16	1.93	1.99	2.03
	20	2.20	2.81	2.24
		±0.22	±0.26	±0.05
B. Day of application of non-nitrogenous nutrients	0	1.85	2.34	1.96
	4	1.82	2.31	1.94
	Never	1.17	1.21	1.82
		±0.19	±0.26	±0.04
C. Day of nitrate application	4	1.80	2.21	1.98
	8	1.43	1.69	1.84
		±0.16	±0.21	±0.04

(Table 4.10 A). Delay in the application of non-nitro-:genous minerals to day 4 had no significant effect, but withholding these minerals completely significantly re-:duced the log dry weight of both tillers and the plant (Table 4.10 B). From results of previous experiments (Figs. 4.8, 4.9; pages161 and167), the effect on tiller buds was to be expected; however, a significant effect on plant dry weight was unexpected and probably reflects the greater accuracy of the large factorial experiment, and the analysis possible by the analysis of variance. There were also significant effects on log dry weight of both tillers and plant when nitrate application was de-:layed from day 4 to day 8 (Table 4.10 C). Thus the main effects of time of harvest, and of time of application of nitrate and of non-nitrogenous minerals, were of simi-:lar statistical significance for both the tillers and the plant.

The various interaction effects, summarised in Tables 4.9 and 4.11 indicate different patterns of growth in the tillers and the plant over the period day 8 - 20. There was a significant interaction ($p = 0.01$) for the plant between time of harvest and the date of nitrate applica-:tion (Tables 4.9 and 4.11 A); the data indicate that the difference in dry weight between plants supplied with nitrate on day 4 and those supplied on day 8 became greater at the later harvest dates (see also Fig. 4.10, G - I). Data in Table 4.11 A suggest that this interaction affected plants up to day 16; from day 16 to day 20 the relative growth rate was similar in both sets of plants. This/

Table 4.11 Log dry weights of tillers (µg) TC and T1, and the plant (mg) to show interaction effects in the factorial experiment.

A. For Plant. Interaction between time of harvest and day of nitrogen application.

Day of nitrogen application	4	8
Harvest Day		
8	1.60	1.56
12	1.85	1.71
16	2.14	1.93
20	2.34	2.14

B. For Tiller TC. Interaction between time of harvest and day of application of non-nitrogenous minerals. Results for T1 are shown in parentheses.

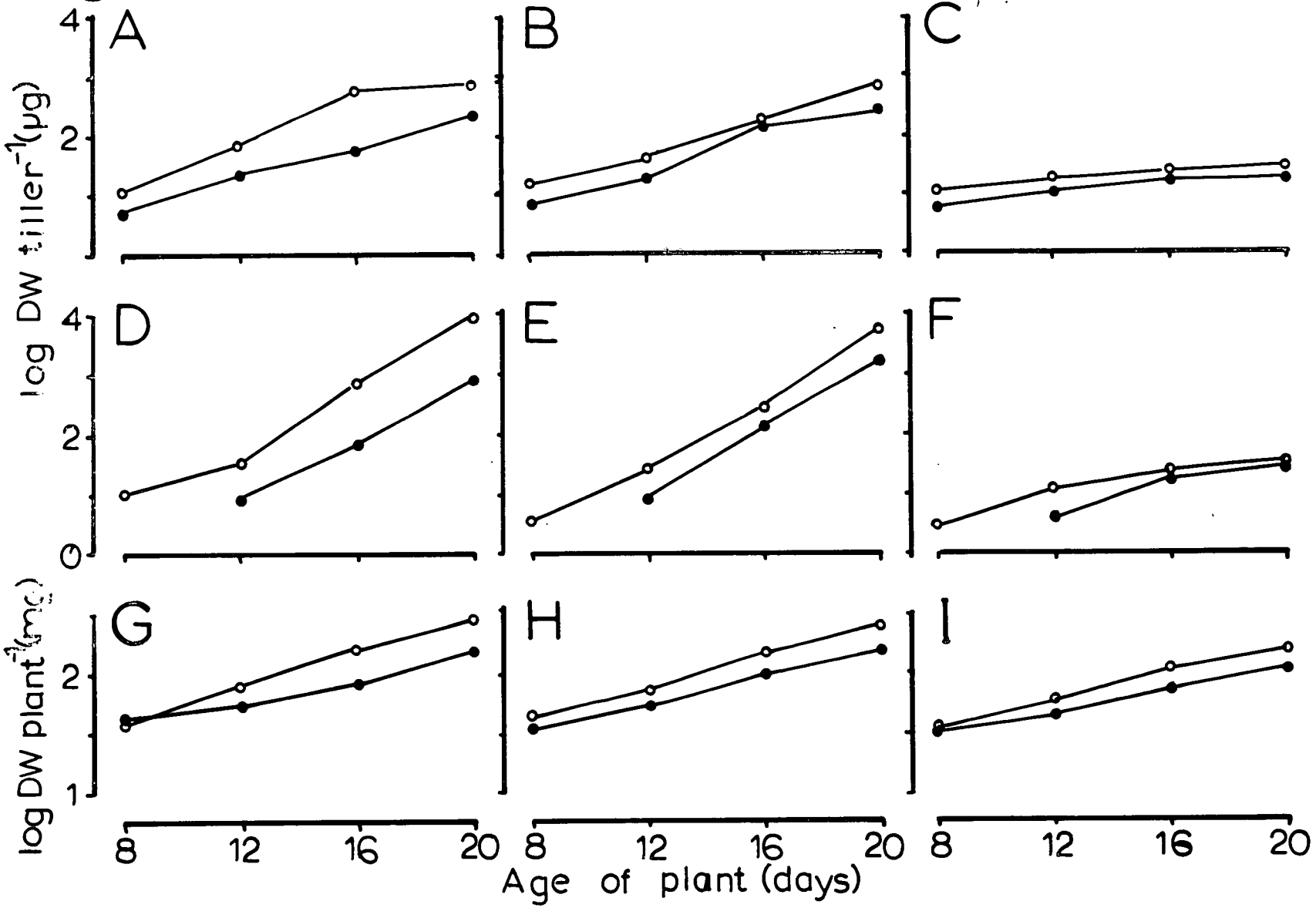
Day of application of non-nitrogenous minerals	0	4	Never
Harvest Day			
8	0.90	1.01	0.91
12	1.60 (1.22)	1.44 (1.16)	1.13 (0.82)
16	2.27 (2.36)	2.21 (2.29)	1.30 (1.32)
20	2.63 (3.45)	2.63 (3.50)	1.36 (1.49)

C. For Tiller TC. Interaction between day of application of non-nitrogenous minerals and day of application of nitrate. Results for T1 are shown in parentheses.

Day of application of non-nitrogenous minerals	0	4	Never
Day of nitrate application			
4	2.15 (2.78)	1.97 (2.55)	1.28 (1.31)
8	1.55 (1.90)	1.67 (2.08)	1.07 (1.11)

Figure 4.10 The effect of application of non-nitro-:genous minerals on day 0 (A, D, G), day 4 (B, E, H) or never (C, F, I), and of nitrate on day 4 or 8 on growth in dry weight of tillers (µg) TC (A, B, C) and T1 (D, E, F), and the plant (mg) (G, H, I).

Fig 4.10


A
B
C
D
E
F
G
H
I
log DW tiller^{-1}(µg)
log DW plant^{-1}(mg)
4
2
0
1
8
12
16
20
Age of plant (days)
∘ = N appln. day 4
• = N appln. day 8

This interaction was not found for either of the tillers over the period day 8 - 20; i.e. the rates of growth of both TC and T1 were similar in both sets of plants supplied at different times with nitrate (see also Fig. 4.10, A - F). However, on day 4, the day of initial nitrate application, there would have been no differences in tiller bud size in plants of the different treatments; therefore, theo-:retically, an interaction would have been expected over the period day 4 - 8 after planting, with tillers having a lower growth rate in plants supplied with nitrate on day 8 than those supplied on day 4. The small size of the tiller buds over this period, and the limited space available for growing plants prevented this result being shown experimentally. The data indicate that relative growth rates were similar in the two nitrate treatments from day 16 and day 8 onwards in the plant and tillers respectively. From Fig. 4.10 it is clear that the dry weight of tillers and plant were always heavier in plants supplied with nitrate on day 4 rather than day 8.

Other interactions were found only for the tillers, and not for the plant. The first of these was the inter-:action between the time of harvest and the application of non-nitrogenous minerals (Tables 4.9 and 4.11 B). For both tillers there was little difference in log dry weight as a result of delaying application of non-nitro-:genous minerals to day 4; however when non-nitrogenous minerals were not supplied at all dry weight increase between successive harvests consistently declined (see also Fig. 4.10). That a similar interaction was not found/

found in the case of the plant gave further evidence that the plant was affected less severely than the tillers through treatment withholding non-nitrogenous nutrient application; Fig. 4.10 I shows that even in the absence of non-nitrogenous minerals the plant continued to increase in dry weight up to the end of the experiment. This re-:sult confirmed data obtained in earlier experiments, discussed in Section III of this chapter (see also Figs. 4.8 and 4.9).

The second interaction significantly affecting the tillers, but not the plant, was between the day of non-:nitrogenous nutrient application, and that of nitrate application (Tables 4.9 and 4.11 C). There was a small difference in dry weights of tillers from plants treated with nitrate on day 4 compared to those supplied on day 8 when non-nitrogenous nutrients were not supplied; how-:ever, with non-nitrogenous minerals supplied on day 0 the difference in tiller dry weights between plants having the two nitrogen treatments was much greater. These results indicate that tillers responded best to an early application of nitrate when the other mineral nutrients had been supplied already.

To summarise, the main effects in this factorial experiment were similar for both tillers and plant, but the interactions were different, indicating a difference in the response of tillers and the whole plant to varia-:tions in the nutrient treatments. The plant was affected less than the tillers when non-nitrogenous minerals were not supplied, and its growth rate was lowered over a longer/

longer period than that of either of the tillers when nitrate application was delayed to day 8.

This experiment was repeated with an exactly similar design, but with the third set of samples harvested on day 15 rather than 16, and showed the same main effects and interactions, confirming those just presented.

(ii) Further factorial experiments

The final two experiments in this section were designed to examine, again factorially, applications of both nitrate and non-nitrogenous minerals on days 4 and 8, or 4, 8 and 12, thus increasing the time span of applications over that already investigated in the preceding experiment.

(a) In the first of these experiments applications were made on days 4 and 8 only, giving 4 treatments. A second application of all nutrients was made on day 11, so that from that date all treatments had received identical amounts of nutrients.

In the experiments so far described harvests have been carried out on particular days irrespective of the stage of development of the plant. Thus if treatment affected, for instance, the dates of appearance of leaves on the mainstem, the size and possibly the number of leaves visible at the time of harvest would have been different in plants of the different treatments. In McIntyre's experiments on axillary bud growth in Agropyron repens (McIntyre, 1965), harvests were made on plants of the various treatments having the same number of leaves per mainstem, so that plants having different treatments were/

were at the same stage of physiological development, but different in age at harvest. To check that there were no major differences in the two methods of harvesting - either on a particular day or at a particular stage of leaf development - two harvests were carried out in the present experiment. The first of these was made on the day of appearance of the third leaf on the mainstem, which was itself affected by nutrient treatment, the leaf appearing earlier with early nutrient application, with the other harvest made on day 18. Each sample comprised 11 plants at both harvests, and analyses of variance were carried out on each set of data; these results are pre-:sented in Table 4.12.

In the samples harvested on day 18 (Table 4.12 A) no significant effect of delaying the application of non-:nitrogenous minerals from day 4 to day 8 was found for any of the tillers TC, T1 or T2, or for the plant, but delay in application of nitrate to day 8 caused a signifi-:cant reduction in all log dry weights on day 18. There was therefore a greater effect of delay in nitrate application than of delay in application of non-nitroge-:nous minerals.

The mean date of appearance of the third leaf was day 13.5 in the two treatments supplied with nitrate on day 4, and days 15.4 and 15.2 when the treatments supplied with nitrate on day 8 were given non-nitrogenous nutrients on days 4 and 8 respectively. In the plants harvested on the day of appearance of the third leaf similar sig-:nificant effects on plant dry weight to those just described/

Table 4.12 Log dry weights of tillers (µg) TC, T1 and T2, and the whole plant (mg) in plants treated with minerals on days as shown, and harvested either on day 18 or at appearance of the third leaf.

A. Harvest day 18

Day of non-:nitrogenous solution application	4		8		
Day of nitrate application	4	8	4	8	
Structure					LSD (p = 0.05)
TC	2.21	1.69	2.14	1.69	0.20
T1	2.63	2.01	2.74	2.06	0.34
T2	1.83	1.45	1.85	1.33	0.20
Plant	2.33	2.11	2.31	2.14	0.06

B. Harvest at the appearance of the third leaf

Day of non-:nitrogenous solution application	4		8		
Day of nitrate application	4	8	4	8	
Average day of harvest	13.5	15.4	13.5	15.2	
Structure					LSD (p = 0.05)
TC	1.81	1.40	1.61	1.38	0.15
T1	1.80	1.68	1.74	1.51	0.13
Plant	2.06	1.94	2.05	1.96	0.04

described for the day 18 harvest were found (Table 4.12 B). Slightly different effects however were found for the tillers; when non-nitrogenous minerals were supplied on day 4 the delay in nitrate application to day 8 significantly reduced ($p = 0.05$) the dry weight of TC, but the effect on T1 just failed to reach a significant level. When non-nitrogenous minerals were supplied on day 8 both TC and T1 had significantly lower dry weights in plants supplied with nitrate on day 8 compared to those supplied on day 4. Thus, delay in the application of nitrate again clearly affected tiller bud dry weight. The effect of delay in application of non-nitrogenous minerals in plants supplied with nitrate on either day 4 or day 8 was less clear. For TC the dry weight was significantly lower when non-nitrogenous nutrient application was delayed to day 8 in plants supplied with nitrate on day 4; For T1 a significant effect was found when non-nitrogenous nutrient application was delayed to day 8 in plants supplied with nitrogen on day 8.

Thus, for the plant, similar effects of nutrient treatment were found when plants were harvested either on day 18 or at the appearance of the third leaf; delay in nitrate application caused a significant lowering of plant dry weight, whereas delay in the application of non-nitrogenous minerals to day 8 had no effect. Tillers showed similar results at both times of harvest to those for the plant when nitrate application was delayed; but delay in non-nitrogenous minerals application had significant effects in two out of four comparisons when plants were/

were harvested at the appearance of the third leaf, where-:as there were no significant effects of this treatment when plants were harvested on day 18.

This difference in result for the tillers when har-:vest was made at the appearance of the third leaf rather than on day 18 was slightly surprising; third leaves appeared on plants supplied with nitrate on either day 4 or 8 on approximately day 13.5 or 15.3 respectively; there were therefore about 4.5 or 2.7 fewer days' growth between the time of the second application of nutrient on day 11 and the date of harvest, in the plants harvested at the appearance of the third leaf than in those har-:vested on day 18. It seems therefore that an effect of delay in application of non-nitrogenous minerals evident in plants harvested up to about day 15 is not observable in plants harvested on day 18; this difference in res-:ponse may be due to the difference in the period of time between the second application of nutrient and the time of harvest.

Although a slight difference in results has been shown by harvesting at the appearance of the third leaf rather than on day 18 the conclusions are broadly similar, and indicate that both times of harvest give comparable results.

(b) In the second of the experiments varying the dates of application of both parts of the nutrient solution, plants were supplied with non-nitrogenous minerals and nitrate separately, and in all combinations on days 4, 8 and 12. No further applications of nutrient were made, and/

and the samples of each of the 9 treatments consisted of 15 plants.

To simplify the discussion of these results, abbre-:viations for the treatments will be used as follows:- x/y denotes a plant treatment in which non-nitrogenous minerals were supplied on day x, with nitrate application on day y. Thus 4/12 indicates treatment involving appli-:cation of non-nitrogenous minerals and nitrate on days 4 and 12 respectively.

Before proceeding with the analysis of the results some discussion concerning the most suitable date for harvesting this experiment is necessary. In the design of all the experiments described in this thesis, and especially the present one, there was a conflict between various factors:- the numbers of treatments to be examined, and harvests to be made; the numbers of plants in each sample, and the space available for growing plants. In this particular experiment, with a total of 9 treatments being investigated, it was impractical to have more than one harvest; it was decided that this harvest would be on day 18.

After the present experiment had been carried out evidence from the experiment studying the effect of delay-:ing the second application of mineral nutrient solution (see Fig. 4.2, page 132) suggested that a later harvest date would have made the results more meaningful, for the following reason:- increase in dry weight of tiller buds in plants having no second application of nutrient had ceased by about day 17 (Fig. 4.2); thus the plateau value was/

was reached about 13 days after application of the com-
:plete mineral nutrient solution on day 4. Therefore since in the present experiment some of the plants did not receive nutrient until day 12, the harvest should have been made on approximately day 25 to allow maximum tiller response to nutrient application; this argument assumes that tillers respond to nutrient application over a simi-
:lar time period when application is made to plants on day 12 rather than day 4, although in fact evidence pre-
:sented in Fig. 4.1 (page 125) suggests that this is not exactly so. Nevertheless it seems reasonable to conclude that harvesting the samples on approximately day 25 would have produced results easier to interpret than those obtained with the harvest on day 18, and therefore care must be taken to ensure that comparisons made between the treatments are valid, and that any significant effects discussed are of genuine biological importance.

Knowing some of the limitations of this experiment comparison of all the treatments in a single analysis of variance would not have been meaningful; therefore three analyses were carried out, grouping together the treat-
:ments as follows:- 8/4, 4/8 and 8/8; 12/4, 4/12 and 12/12; and 12/8, 8/12 and 12/12. The comparisons of greatest interest were between 8/4 and 4/8, 12/4 and 4/12, and 12/8 and 8/12; all these comparisons were valid with the harvest on day 18, and the results are presented in Table 4.13.

Log dry weights of the tillers (µg) TC and T1, and the plant (mg) in the control, 4/4 treatment were 1.75, 1.70/

Table 4.13 Log dry weights of tillers (µg) TC and T1, and the whole plant (mg) in plants treated with minerals on days as shown and harvested on day 18.

	Day of appln.of non-nitrogenous minerals	Day of appln.of Nitrate		TC	T1	Plant
A	4	8		1.52	1.65	2.10
	8	4		1.53	1.44	2.20
	8	8		1.55	1.69	2,05
			LSD(p=0.05)	ns	0.13	0.05
B	4	12		1.35	1.47	1,90
	12	4		1.68	1.66	2.18
	12	12		1.13	1.32	1.85
			LSD(p=0.05)	0,16	0.14	0.04
C	8	12		1.15	1.41	1.85
	12	8		1.45	1.69	2,02
	12	12		1.13	1.32	1.85
			LSD(p=0.05)	0.13	0.15	0.04
	4	4		1.75	1.70	2.31

1.70 and 2.31 respectively when plants were harvested on day 18. Using all the available data to calculate the rate of exponential growth of the tiller buds in plants having a second application of nutrient solution (Fig. 3.5 page 89) the above weights were found for TC,, T1 and the plant on days 12, 12 and 18 respectively; this experiment therefore confirms a substantial affect on tiller develop-:ment in plants having no second application of nutrient, while the effect on the plant was negligible.

The analysis of the results of the three treatments 4/8, 8/4 and 8/8 is now considered (Table 4.13 A). Plant dry weight was significantly greater in the 8/4 treatment than in either of the others, in keeping with earlier results; tiller TC showed no significant differences between treatments, while T1 was significantly lighter in the 8/4 treatments than in the others. Data from a number of other experiments have shown that tillers in plants supplied with nitrate before non-nitrogenous minerals are heavier than those in plants having the converse treatment; it is thought therefore that this low value of 1.44 for T1 in the 8/4 treatment was anomalous.

Results from the analysis of variance in the 4/12, 12/4 and 12/12 treatments (Table 4.13 B) are rather clearer, and the significant effects between treatments were similar for both tillers and the plant. In the 12/4 treatment both tillers and plant were significantly heavier than in the 4/12 treatment; in the 12/12 treat-:ment both tillers and plant were significantly lighter than in either of the other treatments.

Comparisons/

Comparisons between the 8/12, 12/8 and 12/12 treatments also showed similar effects for both tillers and the plant (Table 4.13 C); in the 12/8 treatment both tillers and plant were significantly heavier than in either of the other treatments, which did not differ significantly from each other.

Growth in dry weight of TC can be compared to that of T1 for plants having the various nutrient application treatments by calculation of the ratio of the dry weight of TC to that of T1 (Table 4.14). This ratio was highest when both parts of the nutrient solution were supplied early, and lowest in plants supplied late with the nutrients. These ratios must indicate that T1 dry weight increase was greater than that of TC in conditions of delayed mineral nutrient application, even though at the time of planting TC is considerably larger than T1 (see page 209).

The analyses of this experiment confirm that delay in application of either nitrate or non-nitrogenous nutrients has an important effect on both plant and tiller growth; delay in nitrate application has a much greater effect than delay in application of the other nutrients, and inhibition of increase in dry weight of TC is relatively greater than that of T1 with delay in application of nutrient. In a number of experiments previously described a greater effect of withholding nutrient application has been shown on tiller growth than on that of the plant (see Fig. 4.2, page 132; Fig. 4.3, page 142); however, in this experiment both plant and tiller growth were/

Table 4.14 Ratio of log dry weight of TC : log dry weight of Tl, in plants given nutrient application as shown, and harvested on day 18.

Day of non-nitrogenous mineral application \ Day of nitrate application	4	8	12
4	1.03	0.92	0.92
8	1.06	0.92	0.82
12	1.01	0.86	0.85

were affected to the same extent.

V EFFECTS OF COMPONENTS OF THE NON-NITROGENOUS MINERAL SOLUTION ON EARLY PLANT AND TILLER BUD GROWTH

Significant effects on early tiller bud growth of delaying or withholding the application of non-nitrogenous nutrients have already been demonstrated; the plant is also affected by these treatments but to a smaller ex-:tent than the tillers.

The major elements contained in the non-nitrogenous nutrient solution are calcium, magnesium, potassium and phosphorus, and in this section experiments are described to investigate whether or not lack of each of these ele-:ments in the nutrient solution significantly affects plant and tiller increase in dry weight.

In the main experiment in this section plants lacking in a supply of either calcium, magnesium, potas-:sium or phosphorus, together with a control set supplied with the complete nutrient solution were harvested on days 12 and 20. The compositions of the nutrient solu-:tions supplied to plants of each treatment are shown in Table 4.15. Samples consisted of 11 plants in every case, and an analysis of variance was carried out on the data from each harvest; the results are presented in Fig. 4.11.

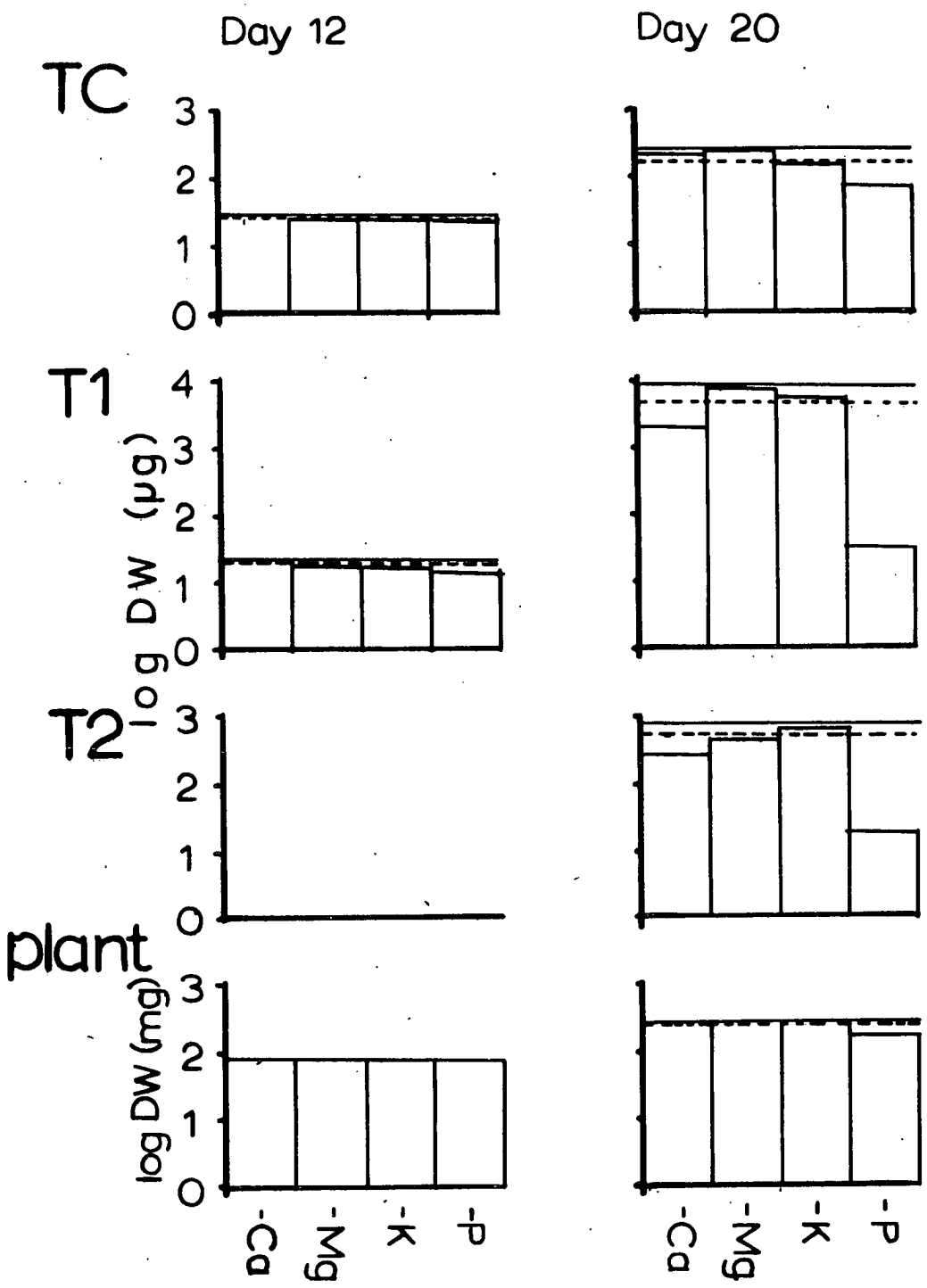
The harvest on day 12 indicated that at this time there were no significant effects of any of the nutrient treatments on growth of either the tiller TC or the plant; tiller T1 was, however, significantly heavier in control plants than in those lacking in either magnesium, potas-:sium or phosphorus. By day 20 all the tillers TC, T1 and/

Table 4.15 Composition of nutrient solutions applied to barley plants to investigate the affect of lack of the major elements apart from nitrogen on early plant and tiller bud growth. Quantities are shown as ml stock solution / litre of solution. Micro-:nutrients and EDTA were supplied in all cases.

Stock Solution	Molarity	Standard	-Ca	-Mg	-K	-P
K_2SO_4	0.5	20	20	20	0	20
KH_2PO_4	0.2	10	10	10	0	0
$CaCl_2$	0.5	10	0	10	10	10
$MgSO_4$	0.4	10	10	0	10	10
KNO_3	1.0	20	20	20	0	20
Na_2SO_4	1.0	0	0	4	0	0
NaH_2PO_4	1.0	0	0	0	2	0
NaCl	1.0	0	10	0	0	0
KCl	1.0	0	0	0	0	2
$NaNO_3$	1.0	0	0	0	20	0

Figure 4.11 The effect of deficiencies of components of the non-nitrogenous mineral solution on dry weights of tillers (µg) TC, T1 and T2, and the plant (mg). Plants were harvested on day 12 or day 20. The control value is given as a continuous line, and the 95% confidence limit from the control value as a broken line.

Fig 4.11



and T2, and the plant were significantly lighter in dry weight in plants lacking in phosphorus than in any of the other treatments. In plants lacking in potassium TC was significantly lighter than in controls; lack of calcium resulted in both tillers T1 and T2, and the plant being significantly lighter than in control plants, and lack of magnesium had a significant effect on dry weight of T2.

The effect of lack of phosphorus was confirmed in a similar experiment using samples of 8 plants, and harvesting on day 20; in this second experiment there was no evidence of an effect of lack of either calcium or magnesium, but tillers T1 and T2 in plants lacking in potassium were significantly lighter than those in controls. Thus only the effect of phosphorus was consistent in the two experiments; it seems that lack of either potassium, magnesium or calcium did not have any significant effect on either plant or tiller growth up to day 20.

A small experiment to investigate further the effect of lack of phosphorus on plant and tiller development was carried out. The growth medium used in this experiment was peralite, which was being considered as an alternative to sand as the growth medium at the time of this experiment. One set of plants was supplied with nitrate, and non-nitrogenous minerals lacking in phosphorus, on day 5; a control set of plants was never supplied with phosphorus, while two other sets were supplied with phosphorus, as potassium orthophosphate (Table 2.1, page 26) on either day 5 or day 11. Harvests of the samples of 8 plants were carried out 6 days after application of phosphorus, and/

and the results are presented in Table 4.16.

Supply of phosphorus on day 5 did not significantly affect the dry weight of either tiller TC or the plant on day 11. There is also no evidence of any effect on T1, although the 95% confidence limits could not be calculated for this tiller at the day 11 harvest, since the buds were too small to be weighed at this time. Supply of phosphorus on day 11 resulted in significantly greater dry weights of both tillers TC and T1 than in plants never supplied with phosphorus, when plants were harvested on day 17. There was, however, no effect of application of phosphorus on dry weight of the plant in the day 17 harvest.

VI DISCUSSION

All the experiments described in this chapter investigated effects of either delay or decrease in mineral nutrient supply on early plant and tiller growth. Considerable effects of these treatments were to be expected in the light of work on effects of nutrient supply on the numbers of tillers appearing above the leaf sheath (Watson, 1936; Aspinall, 1961); such effects were found, both on the numbers of tillers initiated, and on development of buds prior to their emergence from their subtending leaf sheaths. The results given in this chapter are however the first to demonstrate effects of nutrient supply on growth of very young buds.

Both delaying the application, and lowering the amounts of nutrients supplied would obviously be expected to/

Table 4.16 The effect of delaying application of phosphorus on growth in dry weight of tillers (µg) TC and T1, and the plant (mg); 95% confidence limits are indicated, and significant differences are shown by asterisks.

	Day of harvest	11	17	
	Day of application of phosphorus			
TC	Never	1.16 ± 0.116	1.32 ± 0.064	
	5	1.16 ± 0.052	—	*
	11	—	1.76 ± 0.218	
T1	Never	0.60	1.30 ± 0.085	
	5	0.70	—	*
	11	—	2.14 ± 0.303	
Plant	Never	1.76 ± 0.024	2.05 ± 0.047	
	5	1.75 ± 0.052	—	
	11	—	2.06 ± 0.066	

to affect growth of the plant as well as of the tillers. These results were found (for instance, Tables 4.4, 4.10; Figs. 4.6, 4.7), but there is much evidence indicating that growth of tillers was affected to a greater extent than that of the plant in adverse conditions. For instance, increase in dry weight of the plant continued up to day 23 in plants supplied with a single application of the standard mineral solution on day 4, while growth rate of tillers TC and T1 declined from about day 14, with no increase in dry weight being evident after day 17 in plants having this treatment. Application of nitrate or non-nitrogenous nutrients to plants previously supplied only with non-nitrogenous nutrients or nitrate respectively, had greater effects in stimulating growth of the tillers than that of the plant. A third piece of evidence of greater effects on tiller growth than on that of the plant came from experiments investigating the effects of the concentration of nitrogen supplied; when nitrogen supply was decreased from the standard, 14 mg per application, to lower amounts, and growth up to day 20 studied, sig-:nificantly lower dry weights were found for the tillers at an earlier harvest date than in the case of the whole plant. Also, in the experiment in which the amount of nitrogen supplied was decreased, and plants from 11 treat-:ments harvested on day 20, a greater decrease in the amount of nitrogen was required to cause a significant reduction in plant growth than that necessary to decrease significantly growth of T1 and T2. The first factorial experiment also indicated a greater effect on tiller growth/

growth than on that of the plant; the significant interaction effects, which were different for the plant than for the tillers, are all interpreted as showing a greater effect of delay in application of either nitrate or non-nitrogenous nutrients on growth of the tillers than on that of the plant. In some experiments, such as that in which nitrate and non-nitrogenous minerals were applied factorially on days 4, 8 and 12, and also for the main effects of the first factorial experiment, similar significant effects were shown for both tillers and plants; no experiments indicated greater effects on plant growth than on that of the tillers. It can be concluded therefore that in conditions involving either a decrease or a delay in nutrient supply the dominance of the mainstem apex over apices of the axillary buds, the tillers, is increased, resulting in decreased growth of the tillers relative to that of the plant. These results will be further discussed later (page 232) in relation to theories of apical dominance.

Both TC and T1 show small increases in dry weight even in sub-optimal conditions of mineral nutrition, when application of either nitrate or non-nitrogenous nutrients is delayed. On application of whatever nutrient was previously lacking, growth rate is quickly increased, so that a two-phase pattern of growth is obtained, with the second phase being initiated only on application of nutrient. An effect of the concentration of nutrients on tiller bud development has also been shown; this is compatible with the suggestion put forward at the end of Chapter/

Chapter 3 (page 114) that diffusion of assimilate and nutrients must be of great importance to the young tiller bud.

Another point of interest in the results presented in this chapter is the importance of the constituent ele-:ments of the mineral solution for tiller growth. At the beginning of this investigation nitrogen was assumed to be the most important individual element for tiller deve-:lopment; in order to justify this assumption it is necessary for some recapitulation of a number of experi-:mental results. First, it has been shown that although a small amount of bud growth occurs in plants supplied with non-nitrogenous nutrients, but without nitrogen (page 139), greater growth of buds is possible in plants supplied with nitrogen but deficient in non-nitrogenous minerals. Secondly, Dale (1972) showed a significant reduction of photosynthetic rate in the first leaf of barley through delay in nitrogen application, but no effect of deficiency of non-nitrogenous nutrients; over the period of maximum photosynthetic activity in the first leaf, the tiller buds at the coleoptile, first and second leaf nodes are at a very early stage of growth, and therefore would be expected to be affected adversely at this critical stage in their development. Thirdly, work of McIntyre (1972) has also indicated the controlling effects of nitrogen supply on bud growth in Agropyron repens. Fourthly, Gregory (1937) showed that in barley deficiency in nitrogen had greater effects than defici-:encies of any other element in reducing tiller numbers per/

per plant.

The experimental results make it clear that nitrogen is a basic requirement for early tiller growth (Figs. 4.3, 4.6, 4.7, 4.10). It is not certain in what form the nitrogen is important to the tillers, although some deduc-:tions can be made. There are large amounts of free, unconverted nitrate in the first leaf of barley on day 11 and later, in plants supplied with nitrate on day 4 (Dale, Felippe and Marriott, 1974). However, if the second application of nitrate is delayed beyond day 11, the growth of tiller buds is restricted. In view of the small size of the buds at this time, and therefore of their small absolute requirements for nitrogen, it seems pro-:bable that the free nitrate in the leaf is not of any direct use in tiller bud growth, although it is possible that the free nitrate in the first leaf is not available to the tillers. It would be interesting to determine from what stage tillers are capable of reducing the nitrate ion; preparations of nitrate reductase can be made from leaves of barley, and from roots when preparations yield much lower activities (Dale et al., 1974 and unpublished), but it is uncertain whether buds are dependent upon supplies of reduced nitrogen resulting from enzyme acti-:vity in the leaves and roots. To show unequivocally that a tiller bud is capable of reducing the nitrate ion requires demonstration of the presence in the bud of the active enzyme as well as evidence that nitrate reaches the bud; the small size of the tiller buds at an early stage of their development makes these investigations technically/

technically impracticable.

Supply of nitrogen in a reduced form, as the ammonium ion, does not allow greater growth of tiller buds in plants up to three weeks old, and there is evidence of inhibition of growth, as shown also by Dale et al. (1974). However, ammonium is known to have toxic effects on cereals (Jackson and Volk, 1967); these workers suggested that a change in pH in the soil around the roots of plants supplied with ammonium could be one factor contributing to the toxic effect, and Dale et al. (1974) showed that over an 18 day period after application of ammonium, pH fell from 6.0 to 4.0 - 4.2.

The non-nitrogenous minerals have also been shown to be of importance in early bud growth (Fig. 4.10); of the elements contained in the solution only phosphorus has been shown to have a significant effect on plant and tiller growth up to day 20. Significant effects of the other constituent elements were not consistent in the two experiments carried out.

Some discussion of the roles of nitrogen and non-:nitrogenous nutrients in early tiller bud development is necessary. It is quite clear that optimal growth of buds requires a supply of both nitrogen and non-nitrogenous nutrients; only very limited growth of the tillers is possible in the absence of either part of the mineral solution, and, as already stated, it appears that greater growth of tillers is possible with nitrogen supplied in the absence of non-nitrogenous nutrients than in the converse situation.

Where/

Where application of the complete nutrient solution was delayed the rate of exponential dry weight increase in the tillers was lower in plants supplied later with nutrient (Fig. 4.1; Table 4.2). However, in the experiment in which plants were supplied with non-nitrogenous minerals on day 4 and nitrate subsequently there was no evidence of a decreasing rate of tiller bud dry weight increase in plants supplied late with nitrate (Fig. 4.3; Table 4.5). It seems therefore that non-nitrogenous nutrients are essential to the plant early in development in order to maintain the tiller buds in a state in which maximum potential growth is possible when nitrate is supplied later.

Young tiller buds are regions of the plant of high meristematic activity; leaf primordia are being initiated, and growth of these primordia involves both cell division and expansion. Continued development of the apical region itself also requires extensive cell division. Thus for development of tiller buds to be maintained a high metabolic rate is essential, and compounds, especially proteins and nucleic acids must be formed; both nitrogen and phosphorus are essential elements in the formation of proteins and nucleic acids, and phosphorus is important in the chemical processes involved in supplying the required energy. It is not surprising therefore that plants in which either nitrogen or phosphorus is lacking, or present in only suboptimal amounts, are incapable of supporting meristematic activity at all the growing points on the plant. It seems that growth in the tiller buds is inhibited/

inhibited earlier than that at the mainstem apex in conditions of limiting nitrogen or phosphorus.

The major essential elements apart from nitrogen and phosphorus had no significant effects on plant growth up to day 20 after planting, and reasons can be suggested for these findings. Calcium is an important constituent of the cell wall, and in limiting conditions has a marked effect on root growth. Over the period investigated tillers were not producing adventitious roots, and therefore effects of a lack of calcium on tiller growth would not be expected to be visible until later. The main effect of magnesium is in the formation of chlorophyll, and is therefore vital for photosynthesis. Tiller buds must have an extremely low chlorophyll content since over the first two weeks of their development they are surrounded by leaf sheaths. Thus a lack of magnesium, while it could affect photosynthetic rate in the leaves of the mainstem, and therefore the amount of assimilate available to the tillers, would not be expected to affect directly tiller growth, until after emergence of the bud from its subtending leaf sheath. Magnesium is important also as an enzyme cofactor although the absolute amounts required in this way must be very small.

Potassium is important in a number of chemical processes occurring in the plant, although it is not a constituent of essential compounds, as are the other major essential elements mentioned above. It is very mobile within the plant, and this could account for the negligible effect of lack of potassium in plants up to day/

day 20.

The results presented in Chapter 3 showed that rate of growth of TC was always lower than that of T1, and in all the experiments described in this present chapter similar differences between these two tillers have been shown. Further comparison of growth in these two tillers is possible using the data from the experiments on mineral nutrition.

When initial application of the complete mineral nutrient solution was delayed it was found that in both tillers the time of onset of exponential growth was de-:layed; the subsequent rate of exponential growth was affected to a greater extent in TC than in T1. This result is interpreted as indicating a greater inability of TC than T1 to withstand conditions of delayed mineral application. The experiment in which non-nitrogenous nutrients and nitrate were supplied to plants in all possible combinations on either day 4, 8 or 12, gives further evidence of this difference between TC and T1; in this experiment the ratio, log dry weight of TC : log dry weight of T1, was lower for the later applications of nitrate (Table 4.14), indicating that the increase in dry weight of T1 was relatively greater than that of TC.

In Chapter 3 evidence was presented showing that a proportion of TC tillers become moribund at an early stage of plant development, but that there is no similar effect on T1 over the first three weeks of growth. One possible explanation of the greater inhibition of growth of/

of TC than that of T1 when application of mineral nutri-:ent solution is delayed is that the proportion of TC tillers becoming moribund increases as the initial appli-:cation of nutrient is further delayed. An alternative possibility is that delay in the application of nutrient does not increase the proportion of TC tillers becoming moribund, but rather decreases the rates of growth of those TC tillers capable of growth. Evidence from the calculation of the coefficients of variation of samples of TC tillers was used in Chapter 3 to support the idea of some TC tillers becoming moribund at an early stage of plant development. However, calculation of the coeffici-:ents of variation of samples of TC tillers at the final harvests in the treatments having delayed application of nutrient does not help in distinguishing between the above possibilities, since if the proportion of tillers becoming moribund is very large the coefficient of varia-:tion will become small in value. Thus the data obtained in these experiments cannot be used to support either of the two possibilities mentioned above. A further experi-:ment to determine the proportions of TC tillers becoming visible above the coleoptile in samples of plants having progressively further delayed applications of nutrient would be necessary.

A study of the appearance of tillers in the rice plant with delayed nitrogen application (Sekiya, 1963) gives evidence of the tiller buds at successively higher nodes growing well with successively later applications of nitrogen. This evidence supports the idea that the proportion/

proportion of TC tillers becoming moribund early is increased with delay in the initial application of nutrient.

It has been shown that with delayed application of nutrients the growth of TC is limited to a greater extent than that of T1. However, in experiments involving supply of smaller amounts of nitrogen on day 4 after planting, a different situation exists, and T1 is more adversely affected than TC. There are a number of pieces of evidence from different experiments supporting this conclusion; T1 was affected at a significantly earlier harvest date than TC in plants supplied with 7.0 mg or less nitrogen per application, this effect being found in experiments in which either ammonium or nitrate was used as the source of nitrogen. Also, nitrogen supply had to be reduced to 4.2 mg per application to show a significant difference from the control treatment for the TC tiller, whereas for T1 a significant effect was shown with a reduction to only 7.0 mg nitrogen per application. Calculation of the ratio, log dry weight TC : log dry weight T1 (Table 4.17) showed that on a particular harvest day this ratio was generally highest in plants having a low amount of nitrogen supplied, and lowest in plants supplied with 14 mg nitrogen per application. The ratio was also higher in plants supplied with ammonium rather than nitrate. These results indicate that, provided nitrogen is supplied on day 4, TC is able to maintain its growth rate better than T1 when the amount of nitrogen supplied is decreased.

Table 4.17 Ratio log dry weight TC:log dry weight T1 in plants harvested on either day 17 or 18 from 4 experiments in which the amounts of nitrogen either as nitrate or ammonium, supplied to each plant per application was varied

		nitrate treated plants				Ammonium treated plants	
mg nitrogen/ application		14	7	2.8	1.4	0.7	14	7
Experiment	Day of Harvest							
A	18	0.78					0.98	
B	17	0.96		1.05	1.14	1.06	~~0.98~~	
C	17	0.91	0.91	1.01	1.08			
D	17	0.86	0.89				1.07	1.02

CHAPTER 5

RELATIONSHIPS BETWEEN TILLER BUDS, AND MAINSTEM LEAVES DURING EARLY PLANT DEVELOPMENT

Included in the Introduction to this thesis (Chapter 1) is an account of the evidence concerning vascular connections between a tiller and the mainstem; the evidence indicates that the mainstem leaf most closely connected by vascular tissue with a tiller is the one at the next younger node, rather than the one in whose axil the tiller is positioned. However, a number of results obtained during the course of the project have indicated a difference between TC and T1, and it has been suggested (page 114) that this difference may be due to an association, not necessarily involving vascular connection, between the tiller bud and the organ in whose axil the tiller is positioned. The difference in growth pattern between TC, subtended by the coleoptile, and tillers in the axils of the true leaves on the mainstem has been reported previously (Cannell, 1969b; Rawson, 1971; Jewiss, 1972), but no reasons for the difference have been suggested.

It was therefore of interest to find a possible cause of this difference between TC and T1, and to investigate the association between tillers and organs on the mainstem from the time of tiller bud initiation until the formation of vascular links between tiller and mainstem. Two methods have been used in these investigations; the first, a microscopical examination of both transverse and longitudinal/

longitudinal sections of young seedlings, has enabled the anatomical structure and early development of tiller buds to be studied; other information from this examination includes some data on the tiller plastochron, and on the plastochron for foliar primordial initiation on both mainstem and tillers. The second method used in the study involved application of $^{14}CO_2$ to either the first or second leaf on the mainstem, and estimation of the amount of radioactive label transported from each leaf to the developing tiller buds.

I INITIATION AND GENERAL ANATOMY OF TILLER BUDS, AND THEIR RELATIONSHIP TO THE LEAVES ON THE MAINSTEM

The method of preparation of the sections of barley seedlings up to ten days old has been fully described in the methods section (page 32). Sections were examined, and numbers of tiller buds, and leaves on tillers and mainstem noted; camera lucida drawings of sections showing features of particular interest were made, and the results are shown in Table 5.1 and Fig. 5.1.

(i) Initiation of tiller buds, and leaves on mainstem and tillers

The youngest plant material to be sectioned was 24h old, and at this age 4 leaf primordia were visible on the mainstem apex (Fig. 5.1 A). The coleoptile tiller, TC, with a clearly defined prophyll primordium was also pre-:sent, and T1 was visible in most, but not all plants, as a rounded mass of cells at the base of the second leaf, and in the axil of the first (Fig. 5.1 A). These results are similar to those of Dale, Felippe and Fletcher (1972).

Up/

Table 5.1 Appearance of tiller buds and numbers of leaves on mainstem and tiller buds on seedlings of Proctor barley up to day 10.

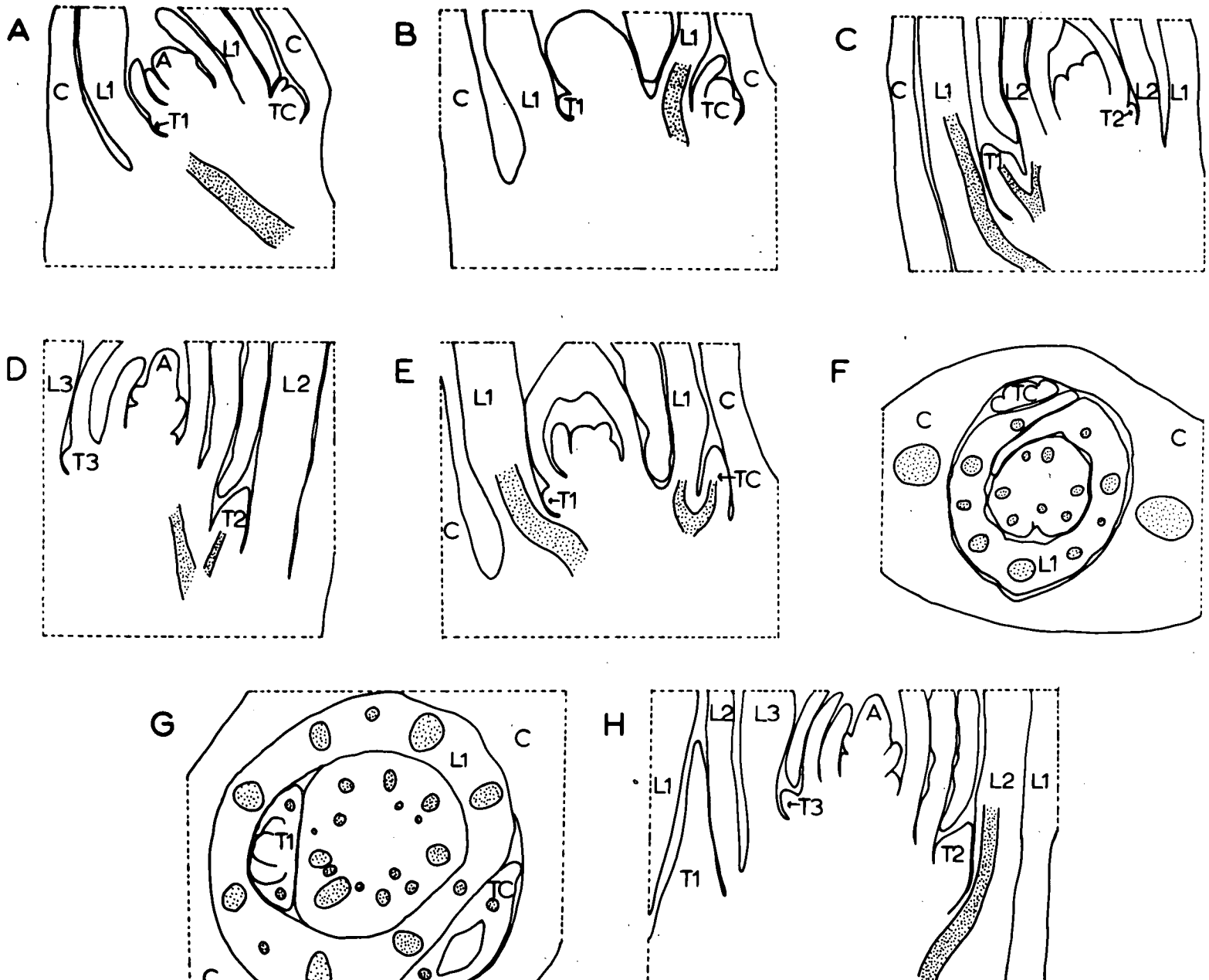
C = coleoptile P = prophyll
* = tiller bud present without prophyll primordium

	Age of Plant	Mainstem	TC	T1	T2	T3
Set 1	24h	C+4	P			
	48	C+4	P+1	*		
	72	C+5	P+1	P		
	96	C+5	P+1	P		
Set 2	4 days	C+5	P+1	P		
	6	C+6	P+2	P+1	*	
	8	C+6	P+3	P+2	P	
	10	C+7	P+3	P+3	P+1	*

Figure 5.1 Camera lucida tracings of longitudinal (A - E, H) and transverse (F, G) sections of young barley plants up to 10 days old. Vascular tissue is indicated by stippled areas. Sections from seedlings 1 (A, F), 2 (B, E), 6 (C, G) and 10 (D, H) days old are presented.

L = leaf; T = tiller; A = mainstem apex.

Fig 5.1


A
C
L1
A
L1
C
T1
TC
B
C
L1
T1
L1
C
TC
C
C
L1
L2
L2
L1
T2
T1
D
L3
A
L2
T3
T2
E
L1
L1
C
T1
TC
C
F
TC
C
C
L1
G
C
L1
T1
TC
C
H
L2
L3
A
L1
T3
L2
L1
T2
T1

Up to 24h from planting seedling development is limited to uptake of water by imbibition, and it can be assumed therefore that structures visible at 24h after planting are also present in the dry grain. By 48h T1 was clearly defined in all plants (Fig. 5.1 B), and in material aged 72h the first, prophyll, primordium was visible on this tiller (Table 5.1). T2 and T3 were visible in the axils of leaves 2 and 3 on days 6 and 10 respectively (Fig. 5.1 C and D), and therefore the plastochron for the formation of primary tillers appears to be about 4 days. This is rather longer than the leaf plastochron of about 2 days reported by Dale *et al.* (1972); the present experiment confirms data of Dale *et al.* showing the presence of the primordium of leaf 7 in seedlings aged 10 days, and again indicates a shorter foliar than tiller plastochron. From the experiment studying the initiation of primordia on mainstem and tillers reported in Chapter 3 (page 76) it can be calculated that during the early stages of growth, before transition to floral development, foliar primordia were initiated approximately every 1.7, 1.8, 1.6, 1.3 and 1.7 days on the mainstem, T1, T2, T3 and T4 respectively. This result indicates a similar foliar plastochron on mainstem and tillers, although as shown in Chapter 3 (page 79), when initiation of floral primordia is included in the calculation it is found that the mainstem plastochron is shorter than that of the tillers.

Between days 1 and 10 TC developed from being a well-defined bud with a prophyll primordium present, to a structure with prophyll and 3 leaf primordia initiated (Table/

(Table 5.1). In the same period T1 developed from being just visible, but with no evidence of a prophyll primor-:dium to a structure also having a prophyll and 3 leaf primordia present (Table 5.1). Thus T1 appeared to develop more rapidly than TC, although TC was still larger than the T1 bud on day 10. In terms of dry weight it has been shown previously that TC remains larger than T1 up to about day 13 - 14 (Fig. 3.5, page 89).

(ii) Anatomical relationships between primary tillers and mainstem leaves.

Vascular connections between tiller buds and adjacent mainstem leaves were investigated using serial sections of the apical region; relevant features are shown in the camera lucida tracings in Fig. 5.1.

The first evidence of a vascular trace in TC was seen on day 2 (Fig. 5.1 E); a connection between this trace and a lateral from the first leaf was also visible on this day in some, but not all cases. The fact that the connection in the bud was not always linked with a leaf trace must indicate that basipetal formation of vascular tissue occurs; this has been shown by Hitch and Sharman (1968) in other grass species. From the longi-:tudinal sections it can be seen that traces in the coleop-:tile were found some distance from the TC bud (Fig. 5.1 F), and no evidence was found for a direct vascular link bet-:ween the coleoptile and TC. In the day 6 material two traces were visible within the prophyll of TC, running laterally along the sides of the somewhat flattened struc-:ture; these traces were not however near the traces in the/

the coleoptile (Fig. 5.1 G). Thus for TC it appears that the initial vascular link with another organ was with the first leaf through a connection to one of the lateral traces in that leaf. It is impossible to say when the vascular tissue in these traces becomes functional; lignified tissue was first visible in the TC vascular strand in material from seedlings 8 days old, but it is not possible from this observation to determine at what stage either xylem or phloem becomes functional in the passage of water and nutrients.

T1 and subsequently formed primary buds were initiated in close association with the base of the leaf at the next younger node; after initiation of a foliar primordium activity of lateral meristems results in growth of the primordium until it encircles the apical region. Where the two meristems meet, at the opposite side of the axis to the point of initiation of the primordium, a tiller bud is initiated; it follows that although a tiller is positioned in the axil of a certain leaf its initiation appears to be more closely associated with the leaf at the next higher node. The relationships between T1 and the second leaf, T2 and the third leaf, and T3 and the fourth leaf are shown in Fig. 5.1 A, C and D respectively.

Vascular traces were first visible in T1 on day 6, and there was evidence of a link between this trace and one of the laterals from the second leaf on the same day (Fig. 5.1 C); thus a link between T1 and the second leaf was present before the latter became visible on day 8. No studies were made of 5 day old material, and therefore it/

it is impossible to say whether there was basipetal formation of vascular tissue in the tiller prior to the connection with the tissue from the second leaf. No direct link was observed between T1 and the traces from the first leaf, but it was interesting to note the close proximity of a substantial trace from the first leaf passing close to the base of T1; this situation was evident as early as day 2 (Fig. 5.1 E), and at a later stage on day 6 (Fig. 5.1 C). No lignified tissue was visible within the trace in T1 on day 10, the last day investigated.

T2 was initiated in association with the third leaf, but a substantial vascular strand from the second leaf was visible close to T2 (Fig. 5.1 H), although there was no link visible between the vascular tissue of the tiller and that of the subtending leaf. Vascular traces in the T2 bud were visible by day 10 after planting, 4 days after initiation of the bud. No direct link was visible between these traces and those of the third leaf on day 10, giving further evidence of basipetal development of vascular tissue in the buds; it is reasonable to suppose that if older material had been examined a link with the third leaf by vascular connection would have been found.

In summary, the evidence from the anatomical investigation indicates that there is an association both at initiation and later by vascular connection between a tiller bud and the leaf at the next younger node. No direct vascular links were found between a tiller and the leaf in whose axil the bud develops, but large strands run/

run out of the first and second leaves close to the developing buds T1 and T2 respectively. There is no vascular strand running out of the coleoptile close to the developing TC bud, and this difference between TC and the other primary tillers suggests a possible reason for the slower growth of TC than of T1. This finding will be discussed fully at the end of this chapter (page 227).

II TRANSPORT OF ^{14}C-LABELLED ASSIMILATES FROM EITHER THE FIRST OR SECOND MAINSTEM LEAF TO DEVELOPING TILLER BUDS

The results of the anatomical investigation together with other results presented in this thesis made it of interest to determine the proportions of assimilate passing to each tiller from individual mainstem leaves. There are a number of other reports of application of $^{14}CO_2$ to grass plants in order to follow transport of assimilate into tillers (Marshall and Sagar, 1968; St. Pierre and Wright, 1972; Clifford, Marshall and Sagar, 1973) although this is the first attempt to examine the situation in very young tiller buds. Several methods of application of $^{14}CO_2$ to leaves of barley were attempted, and these have been discussed fully in Chapter 2. A diagram of the apparatus finally used is shown in Fig. 2.3 (page 41).

Up to about day 14 the plant is dependent for its supplies of assimilate on photosynthetic activity in the first and second leaves on the mainstem; over this period T1 increases in dry weight at a faster rate than TC, although at the time of planting it is smaller than TC. On about day 14 these two tillers have similar dry weights, and/

and therefore it seemed that a study of the period up to day 14 would be most informative in the investigation of relationships between these tiller buds and the leaves on the mainstem during early growth of the tillers. Application of $^{14}CO_2$ to plants was carried out therefore on days 8, 10, 12 and 14. The second leaf had just appeared on day 8, but was assumed to fix negligible amounts of carbon, compared to the first leaf, on that day. On day 14 the third leaf had just appeared in most plants, and was therefore shaded to prevent assimilation of $^{14}CO_2$ over the time of exposure of the plant to $^{14}CO_2$. Thus on day 8 a single treatment was done, while on days 10, 12 and 14 two treatments were carried out involving uptake of $^{14}CO_2$ by either the first or the second leaf. Uptake in a single leaf was ensured by shading the other leaf; preliminary experiments showed that there was negligible assimilation in the shaded leaf. A disadvantage of this method of application was that the whole plant was exposed to $^{14}CO_2$ within the perspex cylinder, so that assimilation by the leaf sheaths was possible, although it is unlikely to have reached a significant level (Felippe and Dale, 1972). At each harvest every treatment was replicated eightfold.

Preliminary experiments indicated that within 3h of assimilation of $^{14}CO_2$ in the leaves the maximum amount of labelled assimilate had been translocated to the tillers; therefore all harvests were carried out 3h after application. The tillers TC, T1 and T2 were dissected from the plant, and counts per tiller determined as/

as previously described (page 43).

The rate of flow of air over the barley plant exposed to $^{14}CO_2$ (150ml per 1½mins) was great enough to ensure that each leaf was able to assimilate CO_2 at its maximum rate of approximately 1 mg CO_2 per hour (Dale and Felippe, 1972; Blenkinsop, 1974; Metivier, unpublished); this calculation assumed that the concentration of CO_2 in the air was 300ppm, and that lowering this concentration to 200ppm over a period of 1½mins did not appreciably limit CO_2 fixation rate.

In the calculation of the results below, one other assumption has been made - namely that on each harvest day the CO_2 fixed by the first leaf in plants having their second leaves shaded, added to the CO_2 fixed by the second leaf in plants having their first leaves shaded is equivalent to the total amount of CO_2 that would have been fixed by the first and second leaves together in non-limiting conditions, and with neither leaf shaded. On this assumption the proportion of assimilate translo-:cated to each tiller from each leaf could be calculated. Results from the preliminary experiments indicated that the assumption was justified.

The results of this experiment are presented in Fig. 5.2, and Table 5.2, in which 95% confidence limits are indicated. The total number of counts reaching each of the tillers increases in successively older plants, with the exception of TC over the period day 12 - 14 (Fig. 5.2 A); it appears that both TC and T1 had low counts at the day 14 harvest in plants having their second leaves shaded, although/

Figure 5.2 Radioactive counts in tiller buds TC, T1 and T2, 3h after uptake of $^{14}CO_2$ by either the first or second mainstem leaf in plants 8 - 14 days old. Shaded areas indicate counts originating from the second mainstem leaf. Absolute counts are shown in A, and counts from each leaf as a percentage of the total count in B.

Fig 5.2

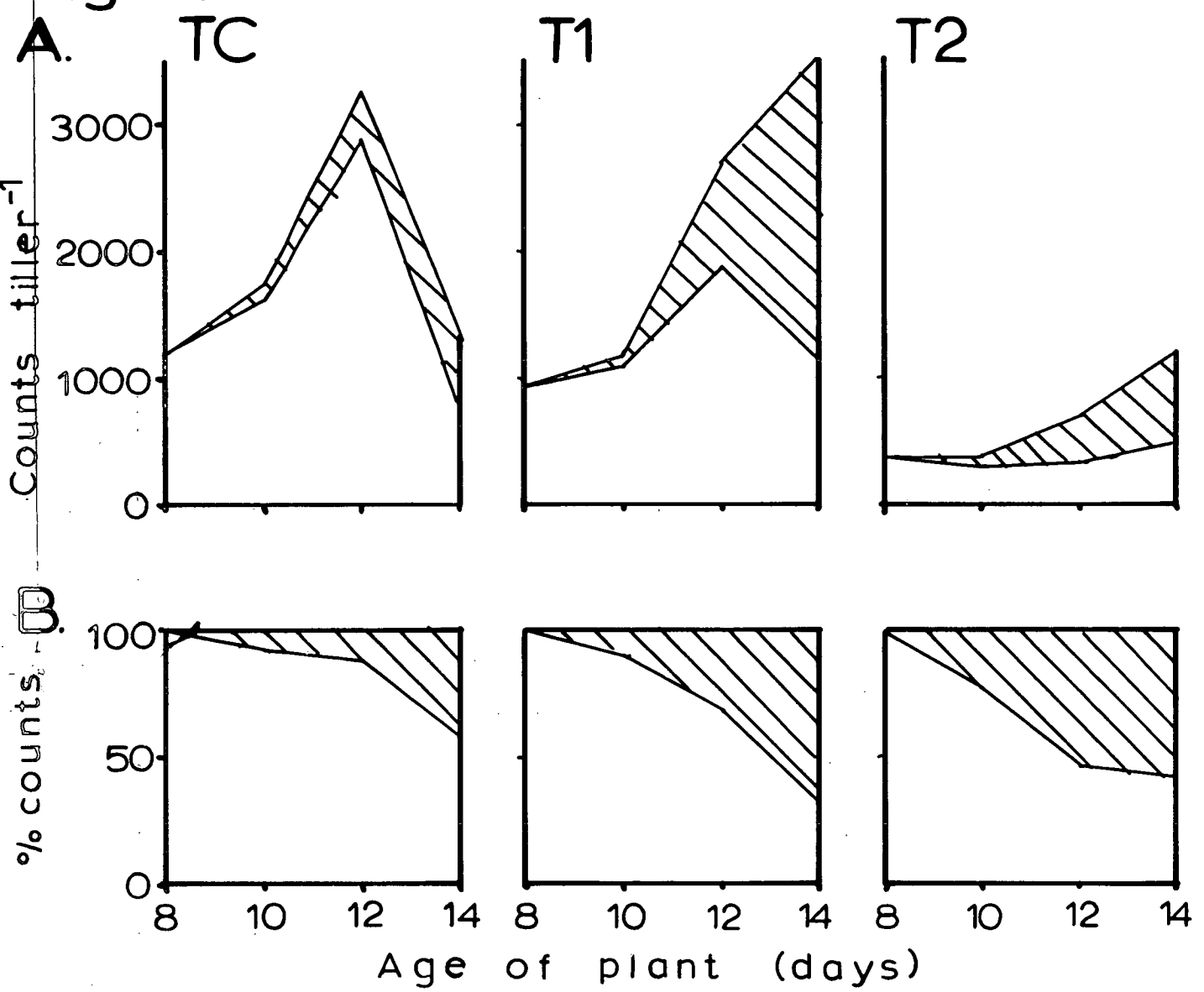

A.
TC
T1
T2
3000
2000
1000
0
Counts tiller-1
B.
100
50
0
% counts
8
10
12
14
Age of plant (days)

Table 5.2 Counts in tiller buds TC, T1 and T2, 3h after uptake of $^{14}CO_2$ by either the first or the second mainstem leaf in plants 8 - 14 days old; 95% confidence limits are indicated.

Day of $^{14}CO_2$ application	Tiller	Counts from First Leaf	Counts from Second Leaf
8	TC	1184 ± 213	
	T1	925 ± 418	
	T2	367 ± 61	
10	TC	1613 ± 378	125 ± 27
	T1	1073 ± 129	116 ± 13
	T2	294 ± 53	89 ± 18
12	TC	2869 ± 787	380 ± 175
	T1	1880 ± 180	866 ± 155
	T2	318 ± 44	363 ± 65
14	TC	764 ± 185	565 ± 271
	T1	1145 ± 253	2383 ± 1162
	T2	489 ± 103	698 ± 252

although the reason for this is unknown. Blenkinsop (1974) showed that the rate of CO_2 fixation in the first leaf on day 14 is only slightly lower than on day 10 or 12; Dale (1972), and Dale and Felippe (1972) showed a rather greater decrease in CO_2 fixation rate on day 14, up to about 30% compared to the day 10 value, but none of these results is of a sufficient magnitude to explain the low counts in tillers TC and T1 on day 14; it seems therefore that the results for counts in both TC and T1 resulting from assimilation in the first leaf on day 14 were anomalously low.

In absolute terms more counts went from the first leaf to TC than to T1, except for the harvest on day 14; a greater number of counts from the second leaf went to T1 than to TC. The total number of counts was greater in TC than in T1 on harvest days 8, 10 and 12, but on day 14 T1 contained more labelled assimilate than TC. These results must be to some extent a reflection of the size difference between TC and T1. At the time of planting TC is much larger than T1, although the rate of increase in dry weight is faster in T1 than in TC. A different batch of grain was used in this experiment than in the ones from which the average dry weight increases were determined (Fig. 3.5) and a small experiment, in which plants grown from the same batch of grain as in the ^{14}C labelling experiment, indicated that the dry weights of buds (μg) on days 8, 10, 12 and 14 were 9, 11, 28 and 197 respectively for TC, and 2, 7, 23 and 204 respectively for T1. These weights are approximately similar/

similar to those obtained on corresponding days in plants grown from the other batch of grain. From these results it was calculated that the counts per µg tiller dry weight on successive harvest days were 132, 158, 116 and 7 respectively for TC, and 462, 170, 120 and 17 for T1. Thus although the intake of counts per tiller was greater in absolute terms for TC than for T1 on days 8, 10 and 12, in terms of the intake of counts per unit dry weight of tiller, values for T1 were higher than those for TC on all the harvest days investigated.

A faster rate of growth of T1 than TC found throughout this project is again shown in the results just presented, since on day 14, when TC and T1 had similar dry weights the counts per tiller µg dry weight were considerably higher for T1 than TC.

The absolute number of counts into T2 was smaller than into either TC or T1 over the period investigated. This tiller was too small to be weighed on days 8 and 10, and on days 12 and 14 its dry weight was 4 and 27µg respectively. Its intake of counts per µg dry weight was therefore 170 and 44 on these two days; these values were higher than those for either TC or T1. However, when tillers T1 and T2 had similar dry weights, on days 12 and 14 respectively the counts per tiller µg dry weight were higher for T1 than for T2, approximately 120 and 44 respectively. It is known from other experiments that the rates of growth of T1 and T2 are very similar (Fig. 3.5) and therefore counts per tiller µg dry weight would be expected to be similar for these tillers when their dry/

dry weights were similar. The fact that the result was lower for T2 than for T1 could indicate that assimilation by the third leaf is important in contributing to the rate of assimilate uptake in T2 by day 14; as stated on page 43 the third leaf was shaded in both treatments on day 14 to prevent it fixing $^{14}CO_2$.

Study of the proportion of counts in each tiller originating from either the first or the second leaf (Fig. 5.2 B) reveals that the proportion of total tiller counts originating from the first leaf was always higher in TC than in T1; also, this same proportion was generally higher in T1 than in T2. The proportion of counts origi-:nating from the second leaf going to each tiller increa-:sed at each successive harvest over the period of the experiment.

It is clear from this experiment that no exclusive association exists between any one of the mainstem leaves and a particular tiller bud. On day 8 the first leaf is the plant's only assimilating organ, and therefore every growing region of the plant must receive assimilate from this leaf in order to continue its growth. As the second leaf expands, the supply of assimilate from this leaf to the rest of the plant increases, and therefore the pro-:portion of assimilate reaching each tiller from the second leaf increases over the period investigated.

There is evidence that T1 receives a consistently greater proportion of its supply of assimilate from the second leaf than in the case of TC; this suggests a closer association between T1 and the second leaf than between/

between TC and the second leaf, and is consistent with the anatomical relationships described earlier in this chapter.

In this section results of only a single experiment have been presented; obviously further experiments are required either to confirm or to deny the findings concerning the passage of labelled assimilates from particular leaves to specific tillers. However, the results of all the preliminary experiments carried out while the technique was being developed support the general conclusions of the final experiment, and it is therefore reasonable to assume that these results are reliable.

III DISCUSSION

The results described in this chapter allow some discussion of the route of nutrient flow into tiller buds during their early development, and of the differences between buds.

The small size of the tiller buds during their early growth makes detailed experimental analysis of the route of assimilate flow into the buds technically difficult. However, certain observations on the differences in growth between TC and T1 make it possible for some suggestions and deductions to be made.

In the experiment in which plants were grown through to maturity (Chapter 3, page 48) it was found that a smaller proportion of TC tillers survived to maturity than T1 tillers, and in the experiments examining dry weights of the tillers in plants grown in standard conditions it has been consistently shown that the rate of increase/

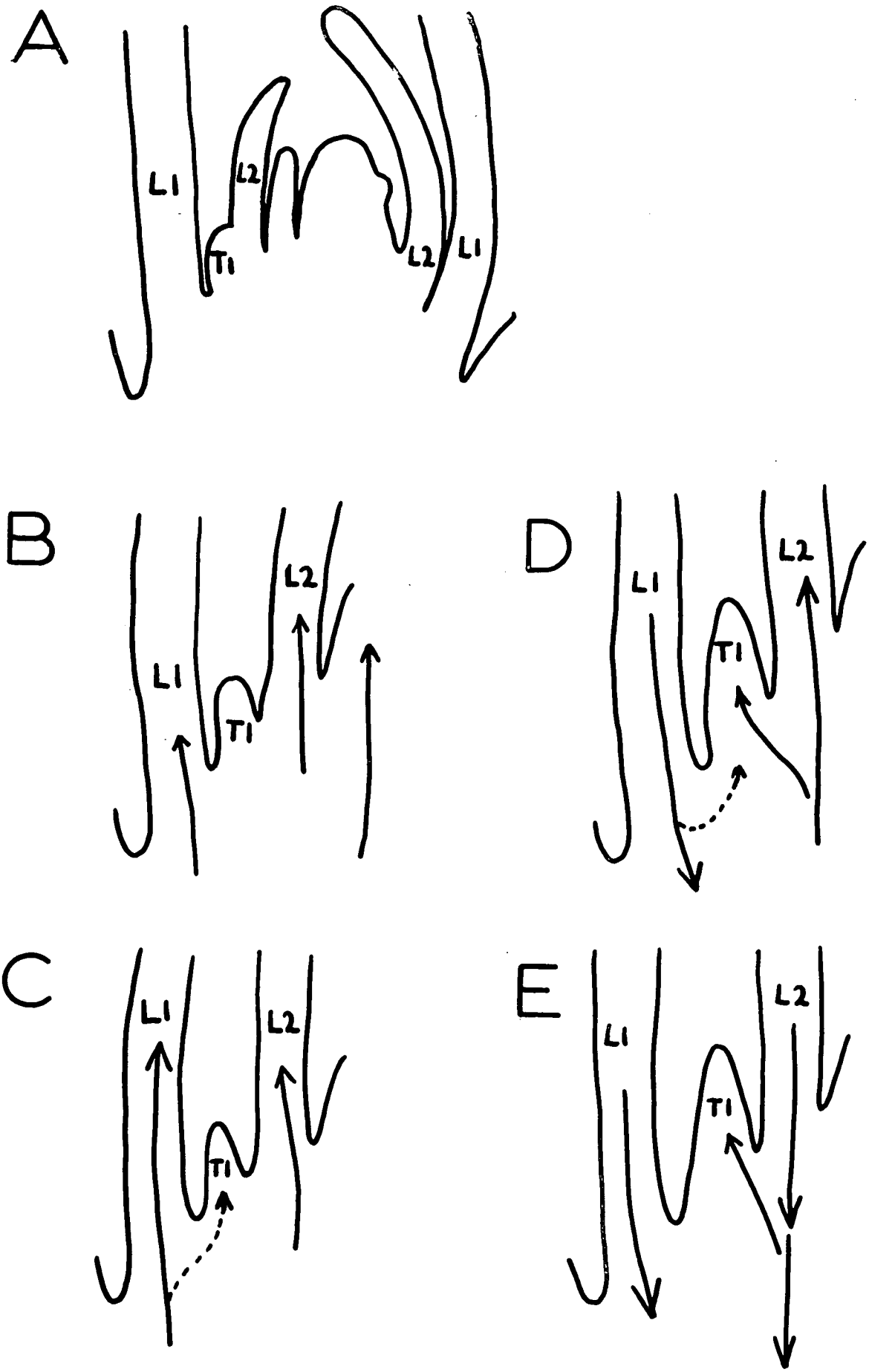
increase in dry weight was greater for T1 than for TC (Fig. 3.5, page 89).

An explanation for these differences is required. It has been mentioned already that in the examination of the vascular connections between tiller buds and leaves at adjacent nodes it was found that there was no direct vascular link between a bud and the leaf in whose axil the tiller is positioned, but that a large trace into the subtending leaves passed very close to the developing buds at the first leaf and higher nodes. No trace into the coleoptile ran close to the TC bud. On the basis of this difference between TC and T1, and other relevant observations the following scheme for the passage of assimilate into T1 is proposed.

Initiation of the tiller bud T1 in barley is closely associated with the activity of the lateral meristem of the developing primordium of the second leaf, so that T1 is formed close to the base of the second leaf (Fig. 5.3 A). Up to day 6 after planting no vascular traces are visible within the tiller primordium, and therefore the passage of assimilate and other nutrients essential to maintain the meristematic activity in the bud must depend on diffusion across undifferentiated cells. The main routes of nutrient flow near the bud must be towards the mainstem apex, and into the first and second leaves, which are both expanding quickly at this time (Fig. 5.3 B). One of the main traces into the first leaf is close to T1; it is proposed that passage of nutrients into the subtending leaf during expansion of that leaf results in some/

Figure 5.3 Diagrammatic representation of early tiller bud development in grasses; ----→ and ⟶ indicate possible routes of flow of assimilates by diffusion or in vascular tissue respectively. For further explanation see text.

Fig 5.3



some leakage of nutrients into the tiller bud (Fig. 5.3 C). From day 6 onwards the first leaf is photosynthesi-:sing, although it does not reach its maximum rate of assimilation until about day 8 (Dale, 1972). Over the period of export of assimilate from the first leaf some materials may leak to the tiller bud from the leaf trace leaving the first leaf (Fig. 5.3 D), but no vascular trace is formed between T1 and its subtending leaf. From day 6 a vascular connection into T1 is present linking up with a lateral trace to the second leaf, although until day 8 the second leaf is not visible; there may then be a flow of nutrients via the vascular trace into T1 as a result of the flow of nutrients into the second leaf (Fig. 5.3 D). The rapid increase in dry weight of the second leaf continues until the appearance of this leaf on about day 8; subsequently the rate of increase in dry weight of this leaf is lower, and the leaf becomes an exporting organ. Because of its vascular connection to the second leaf T1 probably receives the bulk of its supplies of assimilate from this leaf (Fig. 5.3 E); it is clear, however, from the study on the transport of labelled assimilate that there is no exclusive association between a tiller and any one mainstem leaf.

Thus it is proposed that the main association bet-:ween T1 and a mainstem leaf is with the second leaf, both at the time of initiation, and after the formation of a vascular link to this leaf. It is also suggested that the presence of a large trace within the first leaf close to T1 is of importance in the early growth of this tiller.

A/

A similar pattern of development is proposed for all the primary tiller buds initiated subsequently.

Using this scheme for the passage of assimilate into tiller buds three reasons can be suggested for the difference in early growth between TC and T1. First, as already mentioned, there is no trace entering the coleoptile in a comparable position to TC to that entering the first leaf relative to the position of T1. The coleoptile is a small organ, having a final dry weight of approximately 1 - 2 mg, whereas the true mainstem leaves are larger, the first leaf having a mature dry weight of 15 - 20 mg; a second cause of the limited growth of TC compared to that of T1 could be that very much smaller quantities of materials must pass into the coleoptile during its growth to maturity than into the leaves, resulting in smaller amounts of assimilate diffusing into TC than into T1. Thirdly, the first leaf, to which TC is linked by vascular connection, is the plant's only photosynthesising organ over a period of several days, whereas the second leaf is assimilating over a period during which either the first, or the third leaf, or both, are also active. It is probable therefore that there is greater competition for assimilate from the first leaf between TC and the other growing regions of the plant, than between T1 and other regions when the second leaf is exporting assimilate.

CHAPTER 6

GENERAL DISCUSSION

The experimental results obtained in the course of this project are of significance in a number of areas of general physiological and agricultural interest; a discussion of these areas, together with an appraisal of the main points that would benefit from further investigation are presented in this chapter.

I TILLER GROWTH AND EFFECTS OF APICAL DOMINANCE

As a result of the experimental work carried out in this project it is supposed that tiller development occurs through a series of stages which overlap and cannot, except arbitrarily, be completely separated from each other. The first stage is that of initiation of the tiller bud primordium, and it is followed by a post-initiation stage, during which cell division must be occurring at a fast rate, with only a relatively small increase in dry weight. There then follows a period of exponential growth in dry weight, leading to emergence of the tiller bud above its subtending leaf sheath. Shortly after emergence the rate of increase in dry weight of the tiller becomes lower, and is no longer exponential. Transition of the tiller apex to floral development may either precede or follow emergence of the tiller above the leaf sheath. The final stage of tiller development occurs when adventitious roots are produced at the base of the tiller, allowing growth to be more or less independent of the mainstem. Such a developmental sequence must be common to all graminaceous plants, although the transition from one stage to another

may/

may proceed at different rates, and may be affected to different extents by controlling factors.

In barley it has been shown that initiation of primary tiller buds is unaffected by shortage of mineral nutrients; the reduction in the number of higher order tiller buds initiated in conditions involving shortage of mineral nutrients was due to the limitation in growth of primary buds initiated. It is suggested that either shade treatment of the first leaf, or withholding supplies of essential minerals causes a block in growth between the post-initiation stage and the onset of exponential growth; such an effect would be an explanation of the two phase pattern of increase in tiller dry weight found in the experiments in which either the first leaf was shaded or mineral nutrient supply was delayed. Another treatment known to affect tiller bud growth post-initiation without inhibiting initiation itself is the planting of barley at high densities (Kirby and Faris, 1972). However, under normal conditions of growth, in which there are adequate supplies of carbon assimilate and mineral nutrients to the tiller buds the stages of bud development merge into a continuous process.

Another developmental transition which may be affected by environmental conditions is the transition to floral development; under the conditions used for the experiments in this project transition in T1 occurred after the tiller had emerged, whereas T3 was still surrounded by leaf sheaths at the time it first showed floral primordial structures. Aspinall (1966) has shown that/

that in Proctor barley there is a delay of about 8 days in the appearance of double ridge structures on the mainstem when the photoperiod is reduced from 16 to 8 h; it would be expected that in conditions of shorter daylength more primary tillers would have emerged from their subtending leaf sheaths prior to initiation of floral development.

From the results it is clear that the relationships between primary and secondary tillers, and the mainstem involve the phenomenon of apical dominance. Recent reviews of the theories of apical dominance (Phillips, 1969; Guern and Usciati, 1972) have included relatively little information on work with monocotyledonous plants. The main reason for this is that few workers have examined the situation in monocotyledons due to the technical problems of manipulating young axillary buds in these plants. There is no reason to suppose, however, that the control of axillary bud development is basically different in monocotyledons than in dicotyledons, and it is of value to relate work done on tillering to the general theories of apical dominance.

The majority of the work on tillering has investigated numbers of tillers per plant in varying conditions; from the review of the literature in Chapter 1 it is quite clear that a wide variety of environmental conditions affect tillering. The experiments reported in this thesis give evidence of environmental effects on growth of young tiller buds in barley.

First, in plants having their first leaves shaded increase/

increase in the dry weight of TC was minimal until the second leaf started to photosynthesise. Dale, Felippe and Fletcher (1972) showed that although there was a delay in primordial initiation at the mainstem apex in plants having their first leaves shaded, this effect was only apparent from about day 10 onwards, and primordial initiation never ceased. It appears therefore from these two experiments that in conditions of shading the first leaf, the dominance of the mainstem apex over the axillary buds is increased, so that the limited supply of assimilate is utilised to a greater extent at the main-:stem apex than at the axillary buds. Secondly, through-:out Chapter 4, in which results of either delaying the application or reducing the supply of minerals to young barley seedlings were described, it was shown that tiller growth was affected to a greater extent than that of the plant. Thus with alteration in the supply of mineral nutrients the dominance of the mainstem apex over the young axillary buds was increased. Both these results suggest an effect on tiller growth prior to the stage of exponential increase in dry weight. A third result ob-:tained in this project suggests a continuing effect of apical dominance after emergence of the tiller from its subtending leaf sheath; this was the finding that the rates of leaf appearance and primordial initiation were greater on the mainstem than on the tillers, and that there was a hierarchical relationship between stems on the barley plant.

The inhibition of tiller bud growth by either shading/

shading or delay in application of mineral nutrients could be due to direct effects of these treatments on tiller development; for instance, a lack of carbon assimilate being transported to the bud could prevent the bud increasing in dry weight, and a lack of either nitrogen or phosphorus could have a similar effect. Alternatively, the effects of shading, and lack of nitrogen or phosphorus could be indirect, resulting from, for instance, an inhibition of root growth and consequent shortage of metabolites provided from the roots; shading could also have an indirect effect by limiting uptake of nutrients by the roots, or reducing the activity of nitrate reductase, as suggested in Chapter 3 (page 113).

The nutritive hypothesis of apical dominance postulates a direct effect of the supply of assimilate and mineral nutrients on bud growth; since the apex of the mainstem of a plant is a larger sink for nutrients than the apices of the axillary buds it is argued that there is a restriction on growth of the latter when essential nutrients are limiting. However, this hypothesis does not differentiate between a general effect of all the necessary nutrients taken together, i.e. carbon assimilate, nitrogen and phosphorus, as opposed to a specific effect of each individual nutrient, i.e. carbon assimilate or nitrogen or phosphorus.

Both the observations on the effect of shade and of mineral nutrition could be interpreted using the nutritive hypothesis. In plants having their first leaves shaded there must be a shortage in the supply of carbohydrate in the/

the plant, since endosperm reserves are exhausted by about day 8 after planting (Dale and Felippe, 1972), and the shading of the first leaf prevents assimilation until the second leaf has become active photosynthetically on about day 12. The fact that tiller growth in dry weight is minimal, whereas mainstem primordial initiation continues in conditions of shading the first leaf, can be interpreted on the basis of a greater proportion of the available assimilate being directed to the regions of the plant actively growing, such as the mainstem apex, and away from the axillary buds. Tiller growth is restricted to a greater extent than that of the plant when the supply of one or more essential elements is limiting. The mainstem is actively growing from the time of planting, whereas tiller growth begins slightly later; it can be argued that since the region of the mainstem apex including the young developing leaf primordia is larger than the corresponding region on each of the tillers the limited supply of nutrients is transported to the larger sink, i.e. to the mainstem apical region; there is therefore an insufficient supply reaching the axillary buds, which leads to a greater dominance of the mainstem apex over the axillary buds. Evidence from the experiment on apical dome sizes of mainstem and tiller apices showed that initially the mainstem apical dome was larger than the domes of the tiller apices, although this difference did not persist after the transition of the tiller apices to floral development. On day 10 the mainstem apical dome was significantly larger than that of either T1 or T2,/

T2, and at this stage apices of the mainstem, T1 and T2, had 7, 3 and 1 leaf primordia present respectively; the mainstem apical region on day 10 was therefore consider-:ably larger than corresponding regions on the tillers. Evidence from the experiment investigating the effects of a range of concentrations of nitrogen on the growth of tillers and mainstem is consistent with a hypothesis involving a direct effect of nitrogen on tiller growth. This experiment showed that when there was a general shortage in the supply of nitrogen growth of the plant was adversely affected, so that no growth occurred at any of the shoot apices, whereas with adequate supplies of nitrogen there was growth at both the mainstem and tiller apices. There was also an intermediate situation in which supplies of nitrogen were sufficient to allow growth at the mainstem apex, but not at the tiller apices.

Although there is therefore some evidence for a direct effect of nutrients on tiller growth, there are a number of reports in the literature discounting such an effect. Langer (1972) argues that the reduction in tiller growth at the time of stem elongation in grasses cannot be explained simply using a direct, nutritive hypothesis. He points out that 'the depressing effect of stem elongation on tillering does not appear to be a function of mineral supply alone, for it occurs in the presence of abundant nutrients, which even in annual cereals are now known to be taken up well beyond this stage. Only continuous feeding of plants with relatively concentrated solutions, as in Aspinall's experiment (1961),/

(1961), seems to overcome this effect, and there are good grounds for believing that the internal allocation of nutrients is at least as important as their external supply.' Jewiss (1972) has also rejected the nutritive hypothesis as an explanation of the control of tiller bud development. He found that a supply of ^{14}C assimilate was reaching non-growing tiller buds inhibited because of extension and floral development on the mainstem, but that this supply was insufficient to allow tiller bud growth. However, application of the anti-auxin, tri-iodo benzoic acid (TIBA) resulted in a reallocation of supplies of ^{14}C assimilate, and allowed growth of the buds; thus in the untreated plants supplies of ^{14}C assimilate, which must have been present in the plant, were insufficient to allow expansion of the non-growing buds. A direct, concentration effect cannot therefore be used to explain the lack of growth of these buds when no TIBA is applied.

Other reports of effects of growth substances on tiller bud development provide further evidence of an indirect effect of nutrients on tiller growth.

Evidence in favour of a theory of inhibition of axillary bud growth by auxin produced in the mainstem apex has been given by Leopold (1949); but as shown in Chapter 1 (page 8) Leopold's work is of very doubtful significance, and neither Thorne (1962b) nor Aspinall (1963) give support to the direct theory of auxin action. Recently however, evidence of an effect of the anti-auxin TIBA in the control of axillary bud development has been obtained by Jewiss (1972), as mentioned above. Langer, Prasad/

Prasad and Laude (1973) have also shown an effect of TIBA application in increasing the growth of tillers during the early stages of plant development. The results of these experiments involving application of TIBA to plants support a theory of apical dominance involving the action of auxin, but do not necessarily indicate a direct action, as proposed by Leopold (1949).

Application of kinetin to wheat plants has been shown to promote tiller bud development throughout the growth of the plant (Langer et al., 1973). Measurement of growth of the tiller buds was carried out over a short 8 day period after application of kinetin, but Langer et al. do not indicate whether buds released from inhibition continued to grow; in some dicotyledonous plants appli-:cation of auxin is required to maintain the growth of buds released from inhibition by cytokinin (Sachs and Thimann, 1967), although there is no evidence either to confirm or to deny a similar situation in monocotyledons.

One result reported in this thesis is of interest in relation to the role of cytokinins in the control of apical dominance; namely that in barley tiller develop-:ment is seriously affected by a shortage of either nitro-:gen or phosphorus. Unpublished results of Wareing suggest that cytokinin content of leaves of birch, Betula sp., is decreased when supplies of either nitrogen or phosphorus are limiting. It is possible that the reason for the substantial effects of application of nitrogen and phosphorus on tiller development in barley is indi-:rect, and through cytokinin production.

A/

A criticism of all the work mentioned investigating effects of growth substances on tiller development in cereals is that it has involved application of these substances to plants, and in the work of Langer et al. (1973) wheat plants were manipulated and extensively dissected. Tucker and Mansfield (1973) have criticised techniques involving either decapitation of the apex or external applications of growth substances, since these techniques may affect the normal responses of plants to growth substances. Tucker and Mansfield advocate alte-:ring the environmental conditions under which the plant is grown, and correlating the differences in the pattern of growth with concentrations of growth substances found in different parts of the plant. Their experimental work was carried out on Xanthium strumarium; no similar work has been done on graminaceous plants, but one un-:published observation of Dale is of interest in this respect. Two different light regimes were used in a comparison of growth patterns of barley; the first con-:sisted of 16h light supplied by fluorescent and tungsten lights, while in the second 8h light from fluorescent and tungsten sources were followed by 8h tungsten light only. The second regime was therefore relatively richer than the first in far-red light. Both regimes had an 8h dark period within the 24h cycle. Dale found that extension growth of the mainstem was markedly increased in the second regime, with almost complete suppression of the growth of all tiller buds. No estimations of the concen-:trations of various growth substances were made, but it would/

would obviously be of interest to investigate this further to determine whether or not similar results can be obtained in barley, to those of Tucker and Mansfield in Xanthium strumarium.

A further point of considerable interest in relation to the phenomenon of apical dominance was the finding that in Proctor barley the rate of leaf appearance and primordial initiation was faster on the mainstem than on the tillers. There are no other reports of this difference in other cereals, but in pasture grasses it has been shown that the rates of leaf appearance are similar on both mainstem and tillers (Mitchell, 1953; Robson, 1974). In some grasses, however, there is an infinite difference between mainstem and tillers in terms of the rate of leaf appearance, since the tiller buds do not produce any visible leaves; these are the non-tillering species, and 'uniculm' varieties of cereals in which the rate of leaf appearance on the tillers is zero. The tiller buds themselves have been shown to be present in a non-tillering variety of maize (Dale, unpublished), and in a uniculm variety of barley (Bokhari and Youngner, 1971a).

There is therefore a gradation in apical dominance in graminaceous plants, from species in which the mainstem has complete dominance over tillers, through the intermediate type, such as Proctor barley, in which rate of leaf appearance is faster on the mainstem than on the tillers, to the species of pasture grasses in which no difference in the rate of leaf appearance is found between mainstem/

mainstem and tillers. Both uniculm and tillering varieties of cereals are annual plants under normal conditions of growth, whereas pasture grasses are perennial, depending on the production of vegetative tillers at the end of the growing season to allow renewed plant growth the following season. It seems probable that the annual or perennial growth habit is also associated with the apical dominance system. Joffe and Small (1963) reported that in oats there is a tendency towards perennial growth, similar to that found regularly in pasture grasses if environmental conditions are suitable; this observation suggests that the strength of the apical dominance system in grasses can be altered by changing environmental conditions.

Although differences in the degree of dominance of the mainstem apex over the tillers have been shown in different grasses, the mechanism of apical dominance must be basically similar, and therefore a general theory is required to explain the results on the rates of leaf appearance. No data of direct relevance to this discussion are known in the literature, but a number of possible mechanisms can be suggested. It is possible that there is a greater difference in sink size between the mainstem and tiller apices in Proctor barley than in pasture grasses. It is known that in Proctor barley apical dome size is greater on the mainstem than on tillers over a period of time prior to transition of the tiller apices to floral development, but that after this stage dome sizes are similar on both types of stem. It could/

could be that the early difference in dome size causes a difference in sink size resulting in slower growth of the tillers, and that this effect is perpetuated throughout growth. In pasture grasses there appears to be a size difference between the mainstem and the first formed tillers for a much shorter period after planting, than in cereals; it is possible that there is a less substantial difference in apical dome size between mainstem and tillers over the period of transition to floral develop-:ment in pasture grasses than in Proctor barley, and that this results in a similar rate of leaf appearance on the mainstem and tillers.

The mechanism by which such differences between barley and pasture grasses could be produced is unknown. Possibilities include some anatomical differences in the relationships of tiller buds to the mainstem, so that the exponential phase of growth is reached earlier in pasture grasses than in cereals. Tillers in pasture grasses seem to form adventitious roots at an earlier stage of development than those in Proctor barley, and it is possible that the differences in the rate of leaf appearance on tillers relative to that on the mainstem are due in some way to the different pattern of root de-:velopment, possibly by affecting the balance of growth substances within the plant.

Although some of the experimental results obtained in this project can be explained using the direct, nutri-:tive, hypothesis for the control of axillary bud develop-:ment, a number of results are less easy to fit in with such/

such an interpretation; the latter include the findings of other workers on the effects of growth substances, and the fact that in some grasses there is no difference in the rate of leaf appearance on mainstem and tillers, whereas in Proctor barley there is a significant difference. In Chapter 1 (page 11) five suggested mechanisms of apical dominance control outlined by Shein and Jackson (1971), summarising a review of Phillips (1969), were mentioned. Of these five, the nutritive hypothesis, and the direct theory of auxin action can be discarded in relation to tillering. The remaining three -

1. that hormones, particularly auxin, attract nutrients to the point of synthesis or application, and that nutrients are diverted away from lateral buds;
2. that auxin indirectly inhibits growth of lateral buds by the production of some unidentified compound which inhibits lateral bud growth;
3. that auxin at the apex attracts cytokinins from the roots away from the lateral buds, and this lack of cytokin prevents release of the lateral buds from dormancy;

together with the suggestion of Shein and Jackson that it is the balance of hormones which is important, remain as possibilities, and further work is required in order to differentiate between them, or formulate a new hypothesis.

For the first suggestion above to be possible it would be necessary to demonstrate a greater concentration of auxin in the mainstem apex than in the tiller buds; there is no report of such an investigation in the literature.

On the second suggestion it would be postulated that addition of auxin would tend to inhibit tiller growth. However,/

However, application of selective herbicides such as the auxin-like compound 2, 4, dichlorophenoxyacetic acid (2, 4 - D) has no effect.

There is some evidence for the third suggestion above in that Langer, Prasad and Laude (1973), working on wheat, have shown increased growth of individual tiller buds after the application of cytokinin; however, Dale (unpublished) found no increase in tiller growth after spraying barley plants with cytokinin.

Shein and Jackson's suggestion that it is the balance of plant hormones that is important also remains as a possibility.

From the work in this project it can however be stated that any theory put forward to explain apical dominance must satisfactorily explain the substantial effects of carbon, nitrogen and phosphorus nutrition on tiller bud growth.

II RESULTS RELEVANT TO THE DISCUSSION ON THE INTRODUC-:TION OF UNICULM CEREAL VARIETIES ON A COMMERCIAL SCALE

In recent years, as a number of non-tillering, uni-:culm, varieties of cereals have been bred, there has been increasing discussion over whether or not it would be of agricultural significance to introduce uniculm varieties on a commercial scale.

Donald (1968) has proposed an ideotype for wheat. He suggested that to be maximally efficient a wheat plant should have a short, strong stem; few, erect leaves and a large, erect ear having awns. He also pro-:posed that uniculm varieties would be more efficient in the/

the utilisation of available resources than conventional tillering varieties, since in all tillering varieties some tillers grow to a substantial size without yielding any grain, and that the plant should be a weak competitor against individuals of the same species. On Donald's arguments there is no reason to suppose that the ideotype for barley should be different from that for wheat. The main point of interest in Donald's paper with respect to the results described in this thesis is the theoretical advantage in introducing uniculm varieties, and a number of results are relevant.

First, it has been shown that in the conditions of growth used successive leaves emerge on the mainstem, primary and secondary tillers every 5.6, 7.2 and 8.1 days respectively, and that leaf and floral primordia are initiated at a faster rate on the mainstem than the tillers. Theoretically therefore leaves would emerge more rapidly in a population of uniculm plants that in one consisting of mainstems and tillers; it appears therefore that a uniculm variety would be more efficient than a conventional cultivar in the production of leaves.

Secondly, it has been shown that in the conditions of growth used, awns were visible on the mainstem about 6 - 12 days earlier than on the primary tillers; awning on the secondary tillers was later than on the primaries. From this result it appears that there could be two advantages in cultivating uniculm rather than tillering varieties. First, the crop would reach maturity over a shorter period of time making harvesting easier; and

secondly/

secondly, the growing period would be somewhat shortened, so that growth might be possible in a shorter season.

These two results indicate a theoretical advantage of uniculm over tillering varieties for the production of grain in cereals. There are, however, a number of probable disadvantages mentioned in the literature:-

(a) To obtain the optimal number of stems per unit area a considerably higher grain planting density would be required for uniculm than for tillering varieties. Thus a higher proportion of the total barley acreage would be required for the production of grain for the subsequent year's crop if uniculm varieties are used extensively. Greer (1967) reported that approximately 4 - 5% of the total barley acreage in Britain was used for the production of grain to be planted as the next year's crop. Cannell (1969a) suggested that use of uniculm varieties would increase the acreage required to 10 - 11% of the total.

(b) Cannell (1969a) suggested also that the important insurance provided against pest damage and difficult environmental conditions early in growth by tillering varieties would be lost if uniculm varieties are used. Insurance by the use of tillering varieties would be of significance only in conditions in which plants had been damaged in a random way throughout a population. If a large number of plants positioned in a group were damaged even the use of a tillering variety would not offset the loss.

(c) Ears are smaller on tillers than on the mainstem and therefore/

therefore it can be argued that intra-ear shading by component grains within the ear must be less on tillers than on the mainstem, suggesting an advantage of tillering over uniculm varieties (Cannell, 1969a).

(d) It is possible that since tillers are generally shorter than the mainstem better light interception would be obtained in a population of mixed stems than in one in which all the stems were of a more or less uniform height.

(e) The results in this project showed that over the early stages of plant growth tillers had a higher relative growth rate than the whole plant; it could be argued, therefore, that tillers would be more efficient than the mainstem in growth. However, this comparison between tillers and mainstem is of only limited significance, since over the early stages of plant development virtually the whole tiller is composed of meristematic tissue, whereas the majority of the mainstem is composed of non-:dividing cells, which have achieved their maximum dry weight.

To summarise, although there are potential disad-:vantages in the use of uniculm varieties, such as de-:creased disease resistance and the increased acreage re-:quired for the production of the subsequent year's grain for sowing, it does seem that at least theoretically the use of uniculm varieties would increase the overall yield of grain. However, Kirby and Faris (1972) point out that with the agricultural practices presently in use the tillering habit in cereals is valuable. Donald (1968) points/

points out that a population of uniculm plants is not the same as a population of plants of a tillering variety sown at a density preventing tillering; in the case of the latter the density of plants is so great that the plants are 'extremely depauperate' (Donald, 1968), and it has been shown that at very high seed rates the yield per unit area is less than at a lower seed rate (Kirby, 1967; Puckridge and Donald, 1967).

III POSSIBLE FUTURE EXPERIMENTS

In presenting and discussing the experimental results obtained in the course of this project it has been shown that several observations have suggested interesting possibilities for further investigation. These are now outlined:-

(i) Investigation of the reasons for the difference in rate of primordial initiation and leaf appearance rates found in Proctor barley, but not in pasture grasses.

The results in this thesis are the first to show a difference in the rates of primordial initiation and leaf appearance on different stems in a grass plant; it is obviously necessary to investigate these rates in cereals other than Proctor barley to determine whether or not this is a general feature of cereal growth. The effect may have been accentuated through growth in a controlled environment room, with each plant having only a limited rooting volume; it would be of interest to follow growth of pasture grasses in similar conditions, and both cereals and pasture grasses in field conditions to see whether differences between grasses of annual and perennial/

perennial habit were found in a variety of environmental conditions. Detailed comparison of cereals and pasture species during the early part of their life cycles in conditions either causing transition to floral development or allowing continued vegetative growth would also be valuable; such a study might allow changes in apical dome size on mainstem and tillers to be correlated with rates of leaf appearance on different stems. A study of the stage in plant growth at which formation of roots occurs on each stem would also be helpful; such an investigation would determine whether the independence from the mainstem allowed by the formation of adventitious roots on the tillers is a possible reason for the lack of any difference between mainstem and tillers in the rate of leaf appearance in pasture grasses.

(ii) Investigation of differences in growth between TC and T1.

Reasons for the differences in growth of TC and T1 have been suggested during the course of this thesis, and possible routes by which assimilates reach the developing tiller bud from adjacent leaves outlined. Due to the small size of the tiller bud during the very early stages of its development it is technically difficult to investigate the nutritional associations between tiller buds and mainstem leaves; however, it is possible that an autoradiographical study of the apical region would be valuable in determining at what stage of tiller development the suggested nutritional associations occur. Such work could contribute to an understanding of the cause of the/

the difference between TC and T1 in their growth pattern, and therefore of the factors which affect early tiller development.

(iii) Hormonal studies.

No experiments have been carried out during this project on the effects of hormones on early tiller growth, but as a result of this study on tillering two lines of investigation can be suggested. When considering possible ways of studying hormonal control of tillering it is necessary to bear in mind the structure of the grass plant, and the resultant difficulties in treating the mainstem apex without damaging the rest of the plant.

One approach would be to cut away coleoptile or first leaf sheath tissue to expose the young tiller buds TC and T1 respectively; these tillers could then be treated with different growth substances. If such a dis-:section treatment were carried out on young plants about 10 - 12 days after planting the tiller buds would still be very small in size and only a small amount of sheath tissue would need to be dissected away. However, there are disadvantages in such a method; first, in order to expose T1 the median vascular bundle of the subtending leaf would need to be severed, and secondly, application of growth substances to the tiller buds could result in unnatural growth of the plant. The criticisms of Tucker and Mansfield (1973) concerning much of the work carried out on dicotyledonous plants, involving decapitation of the apex and application of growth substances, would be applicable to this technique. Nevertheless, it seems that/

that such a method would be of some value provided that adequate control treatments were carried out, and the results interpreted carefully.

A second approach would be to manipulate environmental conditions by alterations in the quality of the light regime; such treatments would produce plants with different patterns of growth with respect to tiller development. It would then be of interest to attempt a determination of the concentrations of various growth substances in different parts of the plant.

APPENDIX A

Data on the appearance of leaves and tillers, and yields of mainstem and tillers in plants grown in con-:trol conditions, and those having either the first or second leaf shaded. All the data presented in this appendix are from plants grown in a temperature regime of 20°C during the day and 17°C at night.

Tables 1 - 7 in this appendix show results from the 20/17°C regime corresponding to those contained in Tables 3.1 - 3.7 in the text of this thesis, and are referred to on page 65 of the text.

Table 1 Age of plant (days) at the time of appea-:rance of leaves 2 - 10 on the mainstem in control plants, and those having either the first or second leaf shaded.

Mainstem leaf number	Control	Shaded	
		First Leaf	Second Leaf
2	7.8	8.3	8.0
3	13.3	17.0	13.3
4	19.1	22.8	20.1
5	24.0	28.0	25.3
6	29.3	32.9	31.2
7	35.9	39.3	36.8
8	42.8	46.3	42.9
9	49.0	52.3	49.1
10	54.5	58.1	54.0

Table 2/

Table 2 Age of plant (days) at the time of appearance of tillers in control plants, and those having either the first or second leaf shaded.

Tiller	Control	Shaded First Leaf	Shaded Second Leaf
TC	20.5	25.7	24.2
T1	20.1	26.0	25.8
T2	25.2	28.0	26.8
T3	29.6	33.7	31.4
T4	41.7	44.4	41.9
T1.P	27.7	36.4	32.0
T1.1	34.8	-	-
T2.P	31.9	34.6	35.4

Table 3 The numbers of tillers at particular positions appearing per plant in control plants, and those having either the first or second leaf shaded. Numbers in parentheses indicate the numbers of tillers per plant surviving to maturity; 95% confidence limits are indicated.

Tiller	Control	Shaded First leaf	Shaded Second leaf
Primary tillers			
TC	0.8	0.7	0.6
T1	1.0	0.8	1.0
T2	1.0	0.9	1.0
T3	1.0	1.0	1.0
T4	0.9	0.6	0.9
T5	0.1	-	-
Total	4.8 ± 0.5 (3.1)	4.0 ± 0.5 (2.7)	4.5 ± 0.4 (2.4)
Secondary and Higher order tillers from			
TC	0.6	0.4	-
T1	2.1	0.8	0.9
T2	0.9	1.0	1.0
T3	0.3	0.1	0.1
Total	3.9 ± 1.2 (1.2)	2.3 ± 0.9 (0.4)	2.0 ± 0.3 (0.2)
Total tillers per plant	8.7 ± 1.6 (4.3)	6.3 ± 1.2 (3.1)	6.5 ± 0.6 (2.6)

Table 4/

Table 4 Regression coefficients for the rates of leaf appearance on the mainstem and tillers in control plants and those having either the first or second leaf shaded. Correlation coefficients for all the regressions were greater than 0.99.

Stem	Control	Shaded First Leaf	Shaded Second Leaf
M	0.170	0.162	0.172
T1	0.129	0.121	0.130
T2	0.128	0.138	0.134
T3	0.135	0.131	0.149
T1.P	0.122	-	-
T2.P	0.118*	0.109*	0.120

* indicates significantly lower value ($p = 0.05$) of slope of secondary tiller compared to that of the parent primary tiller.

Table 5 Final leaf number per stem, and the number of days to awning (in parentheses) in control plants, and those having either the first or second leaf shaded; 95% confidence limits are indicated.

Stem	Control	Shaded First Leaf	Shaded Second Leaf
M	10.7 ± 0.3 (70.8 ± 2.3)	10.6 ± 0.4 (72.4 ± 2.4)	10.9 ± 0.2 (71.6 ± 2.5)
T1	8.6 ± 0.7 (80.9 ± 3.3)	8.3 ± 0.7 (84.7 ± 3.6)	8.3 ± 0.4 (83.0 ± 3.1)
T2	7.7 ± 0.5 (81.2 ± 1.9)	7.4 ± 0.4 (78.4 ± 2.6)	7.6 ± 0.4 (79.3 ± 2.0)
T3	6.8 ± 0.3 (80.5 ± 1.7)	6.8 ± 0.4 (80.9 ± 2.2)	7.0 ± 0.0 (77.8 ± 1.6)

Table 6/

Table 6 Summary of data on grain yields of mainstem, primary and secondary tillers on plants in control conditions, and having either the first or second leaf shaded. ± numbers indicate 95% confidence limits.

	Control	Shaded	
		First Leaf	Second Leaf
Weights of grain (mg)			
Total Yield per plant (mg)	1676±281	1389±306	1068±276
Total Yield of tillers (mg)	1192±240	883±249	606±233
Yield per mainstem (mg)	484± 78	506± 69	462±135
Yield per primary tiller (mg)	293± 31	293± 38	220± 73
Yield per secondary tiller (mg)	247± 48	230± 32	384±324
Number of grains			
Total grains per plant	47.4±9.3	36.9±8.7	28.4±7.5
Total grains from tillers	34.1±7.6	24.0±7.0	16.9±6.1
Grains per mainstem	13.3±2.6	12.9±2.1	11.5±3.5
Grains per primary tiller	8.4±0.9	8.0±1.1	6.2±2.0
Grains per secondary tiller	6.8±1.0	6.0±3.4	10.0±0
Grain weights (mg)			
Av. wt. per grain from whole plant (mg)	36.1±3.0	38.1±2.6	37.8±2.1
Av. wt. per grain from mainstem (mg)	37.4±3.4	39.6±1.3	40.3±1.9
Av. wt. per grain from primary tiller (mg)	35.7±2.2	37.3±2.4	35.4±3.0
Av. wt. per grain from secondary tiller (mg)	35.1±5.5	41.2±18.1	38.5±32.4

Table 7 Grain yields on primary tillers in control plants. 95% confidence limits are indicated.

	TC	T1	T2	T3	T4
Total grain weight per tiller (mg)	202±555	339±55	255±69	326±58	242±135
Total grains per tiller	4.5±6.4	9.6±1.7	8.2±1.8	9.6±1.6	8.0±2.5
Av. wt. per grain on tiller (mg)	44.0±61.0	36.0±5.0	36.6±3.0	34.1±3.0	31.2±27.1

APPENDIX B

Reprints of articles published using data also discussed in this thesis:-

1. DALE, J. E., FELIPPE, G. M. and FLETCHER, G. M., 1972. Effects of shading the first leaf on growth of barley plants. I. Long-:term experiments. Ann. Bot. 36, 385-95.
2. FLETCHER, G. M., and DALE. J. E., 1974. Growth of tiller buds in barley: effects of shade treatment and mineral nutrition. Ibid. 38, 63-76.

Ann. Bot. 36, 385–95, 1972

Effects of Shading the First Leaf on Growth of Barley Plants

I. Long-term Experiments

J. E. DALE, G. M. FELIPPE, and G. M. FLETCHER

Department of Botany, University of Edinburgh

Extract from

Annals of Botany

Volume 36 No.145

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Effects of Shading the First Leaf on Growth of Barley Plants

I. Long-term Experiments

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Department of Botany, University of Edinburgh

Date received: 2 July 1971

ABSTRACT

Plants of barley were grown under controlled conditions and the first or second leaves covered with tubular shades thus reducing the light intensity at the leaf surface to low levels. Expansion of the shaded leaves was not prevented, but appearance of the next leaf but one and all subsequent leaves on the mainstem was delayed by up to 3 days. Primordia of the first four leaves were present in the dry grain. Shade treatment delayed slightly the initiation of the eighth and subsequent leaves and transition to the double ridge stage at the mainstem apex.

Shading the first leaf caused a temporary reduction in the rate of dry-matter increase of plants, but after 14 days the rate was similar to that of control plants. Smaller effects were found when the second leaf was shaded. Dry-matter production followed two logarithmic phases in the period prior to awn emergence, and rates for the whole plant and for plant parts were similar for control and shaded plants. Thus, apart from the initial perturbation, shading had no effect on growth in terms of rate of dry-weight gain.

Shade treatment did not affect weight per grain or numbers of grain per ear, but over-all yield of grain was significantly reduced since shading delayed the appearance of tillers and also reduced the number of tillers bearing grain. The effect of shade was especially marked on tillers originating on primary tillers. Similar qualitative effects on tiller development were found in an experiment on wheat.

INTRODUCTION

There are many reports that the nutrition of developing cereal grains is dependent on photosynthesis in the grain itself, in other structures of the inflorescence and in the flag leaf (Archbold, 1942; Porter, Pal, and Martin, 1950; Enyi, 1962; Stoy, 1963; Carr and Wardlaw, 1965; Nösberger and Thorne, 1965). Although other leaves contribute little to the grain the possibility exists that the earliest-formed leaves may exert morphogenic effects on development. This is because these leaves are the main photosynthetic organs at a time when mainstem and tiller apices are developing rapidly and undergoing the transition from the vegetative to the floral state. If the activity of these leaves is altered from the usual pattern one might find changes in the subsequent development of the plant.

This possibility was examined by H. K. Porter in unpublished experiments in this department. She covered the early formed leaves on the main axis of young barley plants with tubular shades. The shades were applied from the time of appearance of the leaves and reduced the amount of light falling on the leaves to a

very low level without keeping them in complete darkness. It was thought that this procedure would allow any photomorphogenic reactions which were saturated at low energy levels to occur. Porter showed that while shading the first leaf had large and persistent effects on growth, causing delay in the appearance of subsequent leaves and reduction in tiller number and final grain yield, shading the later-formed leaves had only slight and ephemeral effects.

The substantial responses found in these experiments are interesting and show the first leaf to influence considerably the subsequent development of the plant. We have extended the approach used by Porter and have examined in detail the effects on growth and development of plants in which the first or second leaves were shaded. The present paper reports results of long-term experiments carried through to the time of grain maturation. Our data largely confirm the earlier findings but enable a distinction to be made between the quantitative effects of shading on growth in terms of dry-matter increase, and more qualitative effects on tiller development and grain production. A second, following, paper deals in detail with the contribution of the first leaf to growth and the effects of shading on this.

METHODS AND MATERIALS

(*a*) *General cultural*

Seed of *Hordeum vulgare* L., cv. Proctor, 1969 harvest, obtained from the Scottish Plant Breeding Station, Pentlandfield, was used in most runs. One experiment used wheat *Triticum aestivum* L., cv. Maris Dove and Maris Ranger. Seed was supplied from the Plant Breeding Institute, Cambridge.

Plants were grown singly, in sand, in plastic pots 9 cm diameter at the top, with a volume of 250 cm^3. Experiments were performed in controlled-environment rooms with day and night temperatures of 20 and 17 °C respectively and daylength of 16 h. Warm-white fluorescent tubes and tungsten-filament lamps gave an intensity of 90·8 W m^{-2} in the wavelength range 400–700 nm; this is equivalent to about 2600 fc and 125 cal cm^{-2} 16 h^{-1}.

(*b*) *Mineral nutrition*

In Porter's experiments, conducted with three plants per pot of 12·5 cm diameter, the supply of minerals, and particularly of nitrogen, was chosen to limit tiller production (Gregory and Sen, 1937; Aspinall, 1961, 1963). Nutrient supply was about one-ninth of that found by Gregory and Sen to give maximal dry weight of Plumage Archer barley grown in sand culture. In our experiments nutrient supply and the timing of this was chosen to be close to that used by Porter.

Mineral solution was supplied at weekly intervals from day 4 from planting to day 40; the amounts of minerals supplied per pot on each of the six occasions were: K—43 mg, Ca—10 mg, P—3·2 mg, and Mg—2·9 mg. Nitrogen was supplied as $NaNO_3$ on days 4, 11, 18 (14 mg pot^{-1}) and on days 40 and 45 (7 mg pot^{-1}). Trace elements and iron as the versenate salt were also provided weekly.

(*c*) *Shade treatment*

Shade treatments were applied to the first leaf on day 5 from planting and to the second leaf, where required, on day 10. The shades consisted of paper cylinders 1·4 cm diameter and either 14 cm long, for the first leaf, or 20 cm for the longer second leaf. The cylinders were covered with aluminium foil, open at both ends, and held in position on small wooden stakes. The shades were adjusted at intervals to ensure that later-formed leaves, and tillers, emerged and developed outside them.

The light intensity at different points down shades held vertically was measured, and probably because of internal reflection from the walls of the shade found to be significantly greater than would be expected from an inverse square law relationship (Table 1). Growth of the shaded leaf resulted in a gradually increasing amount of

TABLE 1. *Light intensities (in fc) at different points down the paper shades used in the present experiments.*

Values expected if the inverse square law applied are also given. Shade length was 14 cm and diameter 1·4 cm

Distance from base of shade (cm)	Observed intensity	Expected intensity
11·0	460	48
10·0	82	30
5·0	8	5
1·0	2·2	2

light reaching the tip, but at maximum length (about 11 cm) intensity at the leaf tip did not exceed 460 fc and 1 cm further back had fallen to 82 fc; the value for light reaching the lamina base from the top of the shade was only 2 fc, but some reflection from below would raise this value by a small amount. Since the lamina expands from the base upwards the region of expansion was always in an intensity of less than 20 fc (70 μW cm^{-2}). It was not possible to ensure that all shades were placed exactly vertically, and slight angling of the shades led to minor variations in intensity from the figures discussed above.

Porter used shades of similar dimensions but partially closed at the distal end with a staple; the light intensity inside such shades was difficult to measure with certainty but was less than 20 fc at the top and less than 1 fc at the base. Results of a comparison between the two types of shade are given in the subsequent paper.

(*d*) *Tiller and primordia production*

Primordia production was examined following dissection of the stem apex and longitudinal sectioning using a freezing microtome. Tiller production was followed by daily observations, all tillers being tagged as they appeared with small plastic rings. The numbering method used by Thorne (1962) and Cannell (1969*a*) was used throughout. On this convention a tiller at the coleoptile node is borne at position T_1, a tiller in the axil of the first leaf is borne at T_2 and so on. The exact positions of higher order tillers were also recorded.

RESULTS

1. *Effects of shading on leaves*

Shading the first or second leaf did not prevent considerable growth of those leaves. In both cases length of the lamina of the shaded leaf was up to 10 per cent greater than that of controls, and increases in sheath length of up to 18 per cent were also found. The increases in blade length were, however, offset by a reduction in leaf width so that final areas of shaded leaves was usually close to that of control plants. Shading also reduced lamina thickness by about 10 per cent. Shaded leaves were consistently found to have a significantly lower dry weight than controls. They also tended to become visibly senescent several days earlier than unshaded leaves.

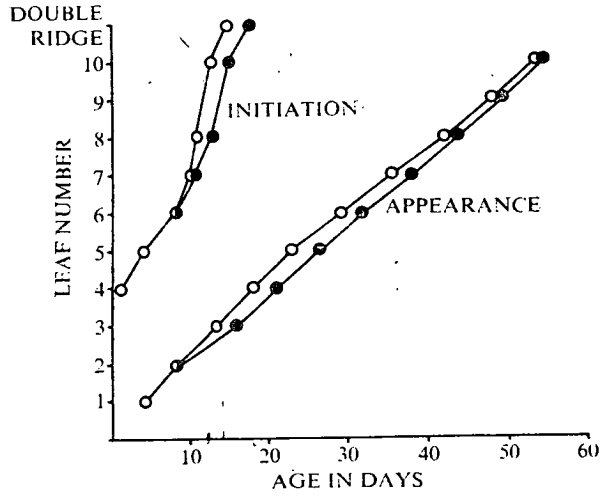

FIG. 1. The time of initiation of leaf primordia, and of leaf appearance for control plants (open circles) and plants with the first leaf shaded (closed circles).

The first leaf, which was about 0·5–1·0 cm long when shading was commenced, reached maximal length of about 10–12 cm on day 8 at which time the second leaf appeared; this in turn reached full length soon after day 14, with some evidence from other experiments that early extension of this leaf is slightly retarded when compared with that of control plants. Shading the first leaf delayed the appearance of the third and all subsequent leaves by 2–3 days (Fig. 1). Shading the second leaf had no effect on appearance of the third but delayed appearance of the fourth and subsequent leaves by 1–1·5 days. For all treatments the time interval between appearance of successive leaves on the mainstem varied between 5 and 7 days, and the tenth leaf was the flag leaf.

Part of the delay in leaf appearance may be attributed to the increase in sheath length of the shaded leaf, since this means that the leaves developing later have to grow longer before emerging. It was thought possible that delay could also be due to a slower rate of primordial production at the stem apex of treated plants. Examinations of the mainstem apex was therefore made to investigate this point.

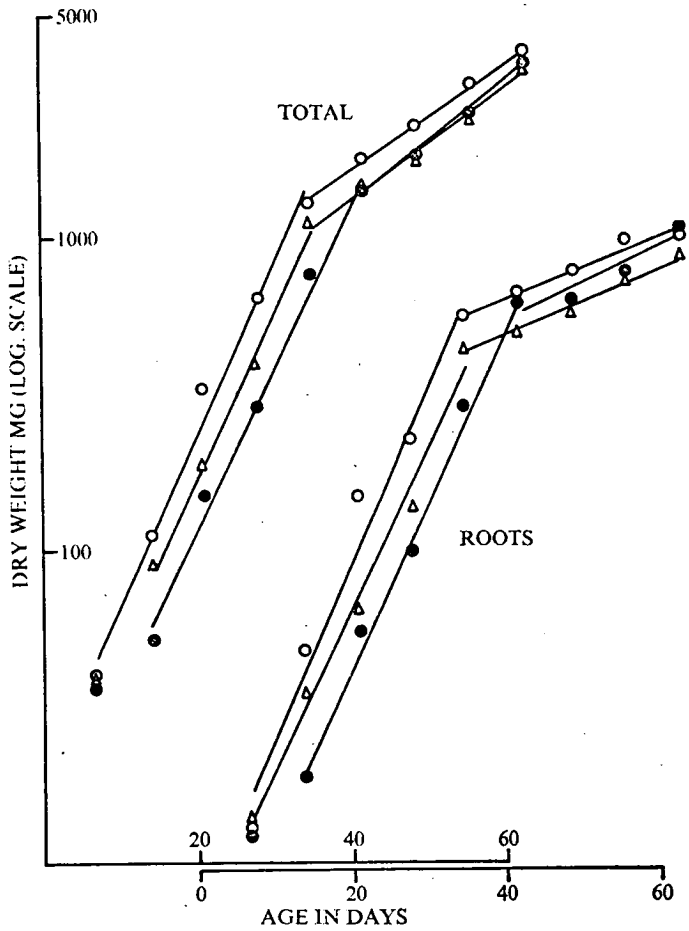

FIG. 2. Changes with time in total dry weight and root dry weight of control plants (open circles) and plants with first (closed circles) or second (triangles) leaf shaded.

The embryo of seeds imbibed for 24 h was found to carry four foliar primordia in addition to the coleoptile (Fig. 1). The effect of shade in delaying appearance of the third and fourth leaves is therefore on structures already present when treatment is started. However, although the plastochron was initially more-or-less constant at about 3 days for control and shaded plants alike, the primordium of the seventh leaf was initiated about a day earlier on control plants. After the initiation of this leaf the plastochron decreased, the reduction being slightly greater for control plants. This difference in plastochron was small, however, and unlikely to be of significance in relation to leaf emergence occurring some 40 days later. The double ridge stage was reached at about day 15 in control plants and 2 days later in the shaded material (cf. Aspinall, 1966).

2. *Effects of shading on plant dry weight*

Plant dry weight was determined at weekly harvests up to day 63. The final harvest was made just prior to awn emergence and some time before maximal plant dry weight was attained. The data are shown plotted logarithmically in Figs. 2 and 3. The linear

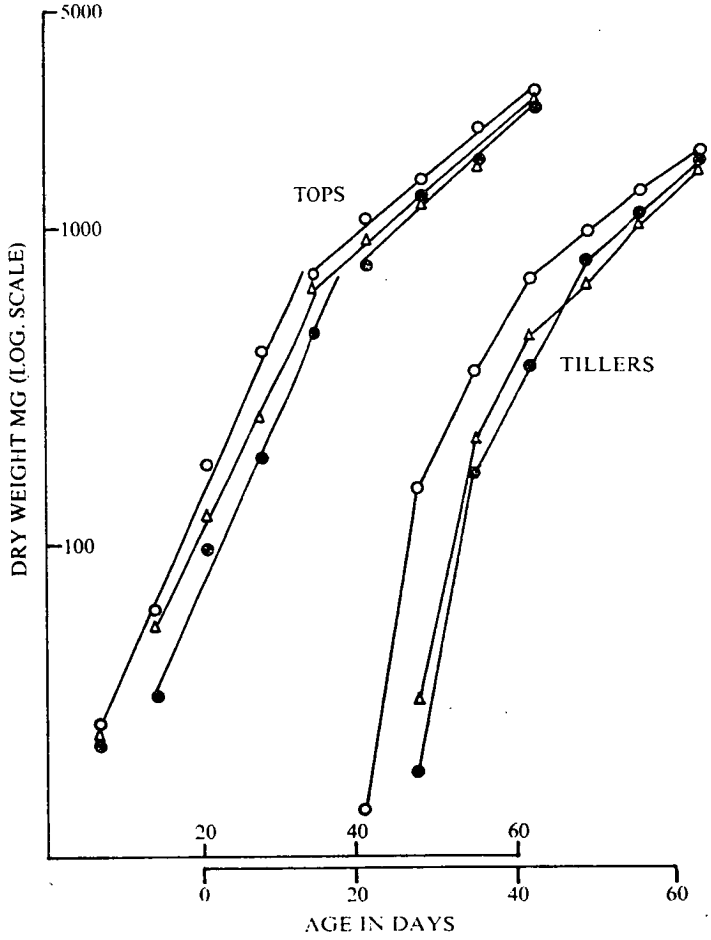

FIG. 3. Changes with time in dry weight of tops and tillers of control plants (open circles) and plants with first (closed circles) or second (triangles) leaf shaded.

curves have been derived and fitted by regression analysis; while this seems reasonable it is recognized that the number of points on which the fit is based is small (Williams, 1964).

Leaving aside the data for tiller weight all the fitted curves show two main phases of exponential growth. For control plants the first phase extended from the first harvest on day 7 to day 35; the second phase from day 35 on shows a smaller exponent. Where the first leaf was shaded the initial exponential phase was delayed for a week until day 14 and continued until day 42; the duration of this phase was therefore similar to that for control plants. Where the second leaf was shaded the data were intermediate between the other two sets. In all cases, for all treatments and for total plant weight and for weights of tops and roots, the slopes of the curves for the first phase of growth were similar. The slopes for the second phase of growth were similar for treatments but were significantly greater for tops as compared with those for roots. This indicates that whereas over the initial phase there was an equal partition of dry

matter between tops and roots, in the second phase tops increased in dry matter at a faster rate than roots. This reflects the increasing diversion of dry matter to the elongating stem and to the developing inflorescence.

The effect of shading the first leaf was to curtail dry-matter production over the period up to day 14 so that both roots and tops were smaller. Subsequently dry-matter production proceeded at the same rate as for the controls so that although at any one harvest shaded plants were smaller this did not reflect a continuing direct effect of treatment, but was merely the consequence of the initial perturbation. The much smaller effect of shading the second leaf probably reflects the fact that the first leaf is productive over the early part of the treatment period and the third leaf becomes productive subsequently, leaving only a short period when dry-matter production is low.

3. *Effects on tillers*

As was expected from the nutrient regime used in this experiment the number of tillers produced per plant was small.

Shade treatment delayed the appearance of tillers (Fig. 3, Table 2). Initial growth-rate of tillers was very rapid, and from about day 35 these organs made a major

TABLE 2. *The time of appearance (days after planting) of primary tillers at various positions*

	T_1	T_2	T_3	T_4	T_5
Control	20·5	20·1	25·2	29·6	41·7
1st leaf shaded	25·7	26·0	28·0	33·7	44·4
2nd leaf shaded	24·2	25·8	26·8	31·4	41·9

contribution to the dry weight of the tops. Insufficient data were available for regression analysis but the shape of dry-weight curves for tillers was similar for control and shaded plants.

Porter found shade treatment to reduce tiller number, and our experiments confirmed this. In a supplementary experiment the position of appearance and development of individual tillers was followed in detail (Tables 2 and 3).

TABLE 3. *The production of tillers at various positions and the number of them yielding grain (in parenthesis)*

	T_1	T_2	T_3	T_4	T_5
			Primary tillers		
Control	0·8 (0·2)	1·0 (0·9)	1·0 (0·9)	1·0 (0·8)	0·9 (0·3)
1st leaf shaded	0·7 (0·3)	0·8 (0·7)	0·9 (0·6)	1·0 (1·0)	0·6 (0·1)
2nd leaf shaded	0·6 (0·1)	1·0 (0·9)	1·0 (0·5)	1·0 (0·7)	0·9 (0·2)
			Second and higher-order tillers		
Control	0·6 (0)	2·1 (0·7)	0·9 (0·3)	0·3 (0)	
1st leaf shaded	0·6 (0)	0·8 (0·1)	1·0 (0·3)	0·1 (0)	
2nd leaf shaded	0 (0)	0·9 (0)	1·0 (0·2)	0·1 (0)	

The first tillers to appear in control plants were those at the coleoptile and first leaf nodes, and shade treatment delayed appearance by up to 6 days. Later-formed primary tillers were delayed to a lesser extent by shading which also delayed appearance of higher-order tillers.

Not all plants produced primary tillers at the coleoptile node (T_1) and there was some indication that shade caused a slight reduction in numbers of tillers at this and the T_2 position. Otherwise the production of primary tillers was only slightly reduced by shade. In contrast, shade very markedly reduced the production of higher-order tillers. This effect was greatest at the T_2 position where control plants produced more than twice as many tillers as the shaded set. Only a small proportion of higher-order tillers produced grain and shade treatment reduced this value further. Shading also reduced the number of ear-bearing primary tillers by a small amount.

TABLE 4. *The effect of shading the first leaf on the percentage of plants of two wheat cultivars showing tillers at the stated positions*

Plants were grown at 20 °C continuously, with 120 cal cm^{-2} $16h^{-1}$ day from fluorescent and tungsten filament lamps

		T_1	T_2	T_3	T_4	T_5
Maris Dove	Control	30	10	100	100	30
	Shaded	20	0	60	90	40
Maris Ranger	Control	*	100	100	50	20
	Shaded	*	0	100	70	10

* Dissection showed all plants to have initiated a bud at T_1, but this did not develop further.

The effects of shade on tillering were also seen in an experiment on the spring and winter wheat cultivars, *Maris Dove* and *Maris Ranger*. The pattern of tillering varied between the two cultivars but shade had significant effects on both, reducing the number of tillers produced by 25 and 33 per cent respectively. The most spectacular effects were to completely suppress tiller production at the T_2 position in *Maris Ranger* (Table 4). In this cultivar tillers are initiated but do not develop at the T_1 position, so that in the shaded plants the first tillers appeared at T_3.

4. *Effects on grain yield*

Table 5 is a summary of tiller production in the main experiment which confirms the general findings from the subsidiary experiment. The reduction in grain-bearing tillers with shade treatment led to reduced grain yield.

Shading did not alter the number of grains borne on mainstem or tillers, nor did it alter the weight of individual grains, although mainstem grains were significantly heavier than those produced on tillers (Table 6). However, the number of tillers bearing grain was significantly greater in the control plants so that grain production per plant was also higher in these plants. Shading the first leaf, by reducing tiller production, reduced grain weight per plant by 13 per cent and grain number per

TABLE 5. *The number of tillers produced per plant, and the number of tillers bearing grain*

	Number of tillers produced	Number of tillers bearing grain
Control	7·8	4·7
1st leaf shaded	5·3	4·0
2nd leaf shaded	5·7	4·3
L.S.D. ($P = 0·05$)	0·89	0·31

TABLE 6. *Grain number and weight for tillers and main axis of control and shaded plants*

Grain number	Control	First shaded	Second shaded	L.S.D. ($P = 0·05$)
Mainstem	18·36	18·54	18·27	n.s.
Per tiller	12·08	11·25	11·82	n.s.
For all tillers	56·28	44·46	50·00	6·20
Per plant	74·64	62·00	68·27	..
Grain weight: (g)				
Mainstem	0·77	0·81	0·80	n.s.
Per tiller	0·42	0·39	0·42	n.s.
For all tillers	1·94	1·55	1·78	0·28
Per plant	2·71	2·36	2·58	..
Average grain weight: (mg)				
Borne on mainstem	41·9	43·7	43·8	..
Borne on tillers	34·8	34·7	35·5	..
Whole plant average	36·3	38·1	37·7	..

plant by 17 per cent. Smaller effects were found where the second leaf was shaded, reflecting the smaller effect of this treatment on tiller production.

DISCUSSION

Our results confirm and extend those of Porter in showing that shading the first leaf of barley seedlings has important effects on growth. Unpublished data for the two cultivars used for tillering studies (Table 4) show first-leaf shading to have similar results on growth of wheat, and it seems likely that the effects of shade are general among cereal seedlings.

It is apparent that although shading the first leaf leads to a rapid reduction in the rate of plant growth, this effect is ephemeral and the over-all pattern of development is, except for tiller production, unaffected by the perturbation. The long-term effects of shading, such as slower emergence of the later leaves and the lower dry weights of treated plants, are the consequences of the early retardation of growth and not of the imposition of a new, qualitatively different pattern of development.

Shading has immediate effects on plant dry weight and it seems reasonable to assume that this is the consequence of a reduced level of photosynthesis in the shaded leaf which is held in a light intensity close to, or below, the compensation point. Evidence to confirm this view is presented in the following paper.

The much smaller effects when the second leaf is shaded are interpreted as due to a continuing contribution from the (unshaded) first leaf so that some newly assimilated carbon continues to be available for growth, even though the second leaf normally contributes the bulk of this. Treatment of the first leaf does not result in a higher compensatory level of activity in the second leaf (Dale and Felippe, 1972). Nor does it prevent expansion of the second leaf, whose growth must therefore either result from photosynthesis within itself, or from a redistribution of dry matter at the expense of other organs, or as seems likely, from both these mechanisms.

Shade has pronounced effects on tillering in both barley and wheat. These effects are permanent and also qualitative in that the pattern of tiller production, as well as the number of tillers developing, differs between shaded and control plants. Cannell (1969*a*, *b*) working with three cultivars of barley showed that the coleoptile tiller (T_1) and the higher-order tillers develop less frequently than other primary tillers. Our results show that the unfavourable conditions imposed by shading greatly reduce the number of tillers produced at these positions. Results from dissection (Fletcher, unpublished) indicate that under the conditions of these experiments the primary tillers at T_1 and T_2 begin to grow exponentially before the endosperm reserves are exhausted and continue to grow using metabolites derived from the first leaf. However, in shade, appearance of the tillers is markedly delayed indicating that treatment curtails the supply of materials for growth. While the rapid growth-rate of newly appeared tillers (Fig. 3) suggests that their vigour is not greatly impaired, the reduction in numbers of higher-order tillers indicates some continuing effect of the shade treatment.

Interpretation of the effects on tillering can only be tentative at this stage. A reduction in tiller development could result from a failure in the supply of elaborated carbon from the shaded first or second leaf. On this basis photosynthetic activity in the first leaf, where the second is shaded, and in the second, where the first is treated, is clearly inadequate to support tiller growth. This implies that although the nutritional requirements of tillers are initially small in absolute terms, they are not met when only one leaf is functional.

Other explanations could be advanced for the failure of tillers to develop. For instance, work with a number of species, including maize and barley, has shown that there are diurnal variations in the level of the enzyme nitrate reductase in leaves; it has also been shown that levels of the enzyme are reduced if the leaves are kept in shade conditions (Hageman and Flesher, 1960). In our experiments nitrogen was supplied as nitrate and it is possible that shade results in a low level of nitrogen assimilation thus leading to a shortage of elaborated nitrogen compounds at the tillers. Alternatively, shade could act indirectly by affecting root growth and activity and hence the supply of available nutrients, including nitrogen, to leaves and tillers. These possibilities, which are being examined further, are attractive in view of the known

sensitivity of tiller development to levels of nitrogen in the growing medium (Aspinall, 1961, 1963).

The importance of the flag leaf and adjacent structures on the nutrition of the developing grain has already been mentioned. The present results make it clear that the grain yield from the plant can be profoundly affected by the conditions under which the young seedling develops since it is in the earliest developmental stages that the future fate of tillering branches appears to be decided.

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Growth of Tiller Buds in Barley: Effects of Shade Treatment and Mineral Nutrition

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ABSTRACT

Examination of the stem apex of *Proctor* barley showed that the bud of the coleoptile tiller, Tc, is probably present in the dry grain and that the bud, T1, carried in the axil of the first leaf is present at or soon after 24 h from planting. Subsequently tiller buds are initiated with a plastochron of about 4 days, this being rather longer than that for the foliar primordia. During the initial phase of bud growth vascular connections are established with the leaf above, but not to the subtending leaf. At some time after these vascular connections are formed and when it has a dry weight of 4–7 μg the bud enters a phase of rapid, exponential growth in dry weight.

Shading the first leaf delays the onset of rapid growth for both Tc and T1, but after a lag period rapid growth commences; this is coincident with development of the second leaf as an organ exporting assimilated carbon.

The phase of rapid growth of tiller buds is delayed when application of either nitrogenous or non-nitrogenous minerals is delayed. Ammonium was found to be less satisfactory as a nitrogen source than nitrate, probably because of toxicity effects. Slight growth of Tc and T1 occurs in presence of non-nitrogenous minerals and absence of nitrogen but growth is greater when nitrogen is supplied in absence of the other minerals, although such growth is substantially less than that found when all nutrients are supplied. The interaction between nitrogen and non-nitrogenous minerals which controls bud growth was not found to affect growth of the parent plant which is, as previously shown, controlled by timing of the nitrogen supply. Another distinction is that higher concentrations of nitrogen and the other minerals are required for maximum growth of the bud than for that of the plant.

Tiller bud growth is interpreted as occurring in two phases. In the first, initiation, phase there is a close association with the subtending leaf, and nutritionally bud and leaf are linked. This phase is followed by one in which the bud is directly connected by vascular traces to the leaf above, which because of this controls bud growth by modulating supply of assimilated carbon and nitrogen, and other minerals to it.

INTRODUCTION

In barley, buds in the axils of the lower leaves on the main stem develop as tillers. Primary tillers, which may themselves produce secondary and higher order tillers, are by definition closely associated with the main stem although their appearance above the sheath of the subtending leaf may not occur for 3 weeks or more following emergence of the main axis. Ultimately, tillering branches produce adventitious roots and may develop into fruiting stems; yield of grain from such branches can be substantial. Tillering is also of major importance in perennial and pasture grasses, and because of the agricultural significance the process has attracted much research. At a more fundamental level, because of the close association with the main stem, tillers are of interest in connection with apical dominance effects (McIntyre, 1971; Jewiss, 1972).

Most published work on tillering has analysed numbers and growth of tillers which have emerged above the subtending leaf sheaths, and which are already substantial in size. As an example we consider the effects of nitrogen supply. Using *Plumage Archer* barley, Gregory and Sen (1937) showed that a ten-fold increase in tiller number occurred when nitrogen supply was increased from 15 mg N pot^{-1} to 1200 mg N pot^{-1}. Aspinall (1961), using *Piroline* barley, found that tiller number was a function of nutrient level and

that where nitrogen supply was high (1100 mg N pot^{-1}) production of tillers was continuous up to ear appearance.

The large numbers of tillers found in high nitrogen regimes must be due to the extensive development of secondary and higher order tillers. This is because the number of primary tillers that can be produced is limited, firstly by the number of leaves on the main stem, and secondly by the onset of stem elongation associated with earing; the number is therefore finite and small and in our experiments with *Proctor* barley (Dale, Felippe, and Fletcher, 1972) a maximum of five or six primary tillers was produced. Yet despite the fact that the major effect of mineral nutrition is on the production of high order tillers, the effect of nutrient supply, especially nitrogen, on growth of the buds of the parent primary tillers has not hitherto been investigated. Indeed, although some descriptive work is available on the histology of initiation of tiller buds in other Gramineae (Sharman, 1945) quantitative data on development of buds in barley prior to emergence are non-existent.

The present paper describes work having three aims. The first of these was to elucidate the developmental anatomy of primary tiller buds, and in particular the vascular connections between these and the adjacent leaves. This has special significance in view of the uncertainty about the role of vascular connections in apical dominance phenomena (e.g. Sachs, 1970; Cutter, 1972). The second aim was to examine the effects of leaf shade treatment on bud growth. Dale *et al.* (1972) demonstrated that tiller number was affected by shading the first leaf of young seedlings and the possibility that growth of the primary tiller buds would be affected seemed likely. The third and most important aim was to investigate the role of mineral nutrients on bud growth. This involved attempts to separate effects due to nitrogen from those due to other mineral nutrients.

METHODS AND MATERIALS

General methods

Barley, *Hordeum vulgare* L., cv. *Proctor*, was grown from grain of the 1970 harvest, obtained from the Scottish Plant Breeding Station, Pentlandfield.

Plants were grown in sand, in a controlled environment at a constant temperature of 20 °C. Photoperiod was 16 h, with irradiance from warm white fluorescent tubes and tungsten filament lamps of 90 W m^{-2} in the wavelength range 400–700 nm.

Shade treatments involved enclosing the first leaf in a paper cylinder covered with aluminium foil, on day 5. Full details of this treatment which suppresses photosynthesis in the treated leaf are given by Dale *et al.* (1972).

Normally, nutrients were supplied on day 4 after planting, and subsequently at weekly intervals. The standard nutrient solution contained elements in the following amounts:

N at 14·0 mg pot^{-1} (as $NaNO_3$)
P at 3·2 mg pot^{-1} (as KH_2PO_4)
K at 43·0 mg pot^{-1} (as KH_2PO_4 and K_2SO_4)
Mg at 2·9 mg pot^{-1} (as $MgSO_4$)
Ca at 10·0 mg pot^{-1} (as $CaCl_2$)
Iron as the versenate salt, and micronutrients.

Other experiments were performed in which time of application of nutrient solution was varied as was also the composition of the solution. Details of these variations are summarized in Table 1.

Tiller nomenclature is the same as that used by Kirby and Faris (1972) and Jewiss (1972) in which the tiller carried in the axil of the coleoptile is designated Tc, and the first, second, and third leaf tillers are designated T1, T2, and T3 respectively.

TABLE 1. *Details of treatments and harvesting procedures in experiments in which mineral nutrition was varied*

Experiment	Day of initial application of solution without nitrogen	Day of initial application of nitrogen	Days of harvesting	Number of plants/ sample
Delay in nutrient application	2, 4, 6, 8, 10, or 12	As opposite	2, 3, or 4—daily up to day 21	10
Delay in nitrogen application	4	4, 6, 8, 10, or 12 with N as either NH_4^+ or NO_3^-; control set with no N applied	3 and 7 days after N application; control set every 2 days from day 5–15	8
Delay in solution without nitrogen application	5, 8, or 11; control set with no solution applied	4	3 and 6 days after application of solution without nitrogen; control set days 5, 8, 11, 14, 17	9
Variation in concentration of applied nitrate	4	4 applying 0·7–14 mg N/ application	Day 20	8

Methods for investigating small tiller buds

To investigate the early growth of tiller buds, samples of up to 10 plants were dissected at two, three, or four daily intervals up to day 32.

The dissection under a Vickers Sterimag microscope involved carefully removing the coleoptile and outer leaf sheaths using a fine needle and watchmaker's forceps, and cutting the tiller buds from the stem using a tool made from a surgical needle mounted in a holder and filed to give a fine cutting edge. Buds were placed on a watchglass or in a small vial and dried at 90 °C; weights were then obtained, using a Sauter Torsion balance for values up to 1 mg. Occasionally plants having two tillers at the coleoptile node or showing other symptoms of abnormal growth were found, and these were excluded from the sample.

Material for sectioning was dissected from the plant, and embedded in paraffin wax, having been pre-stained in safranin to aid orientation in the block. Longitudinal and transverse sections were cut at a thickness of 10 μm using a Beck Rotary microtome. After further staining with safranin, and on occasions with light green, sections were examined to determine the number of leaf primordia and patterns of vascular connections in buds up to 10 days old.

RESULTS

Initiation and general anatomy of tiller buds

It was known that by 24 h after planting the embryo of *Proctor* barley carried the coleoptile and primordia of the first four main-stem leaves (Dale *et al.*, 1972). Whether the imbibed seed also carried the initials of tillers was examined using sections from two sets of plants.

It was not practical to examine plants less than 24 h old, but material of this age (Fig. 1E) showed clearly the presence of the coleoptile tiller, Tc, which already carried the first, prophyll, primordium. Very little visible development of the main-stem apex occurs up to 24 h from planting and it can be assumed that the presence of so well developed a bud is indicative of its presence in the dry grain. There remains doubt as to

whether T1 had been initiated by 24 h; in most but not all plants it appeared to be present as a rounded mass of cells in the axil of the first and at the base of the second leaf. By 48 h T1 was invariably recognizable, although the prophyll primordium was not visible until 72 h (Table 2). T2 and T3 appeared in 6- and 10-day-old material respectively, when

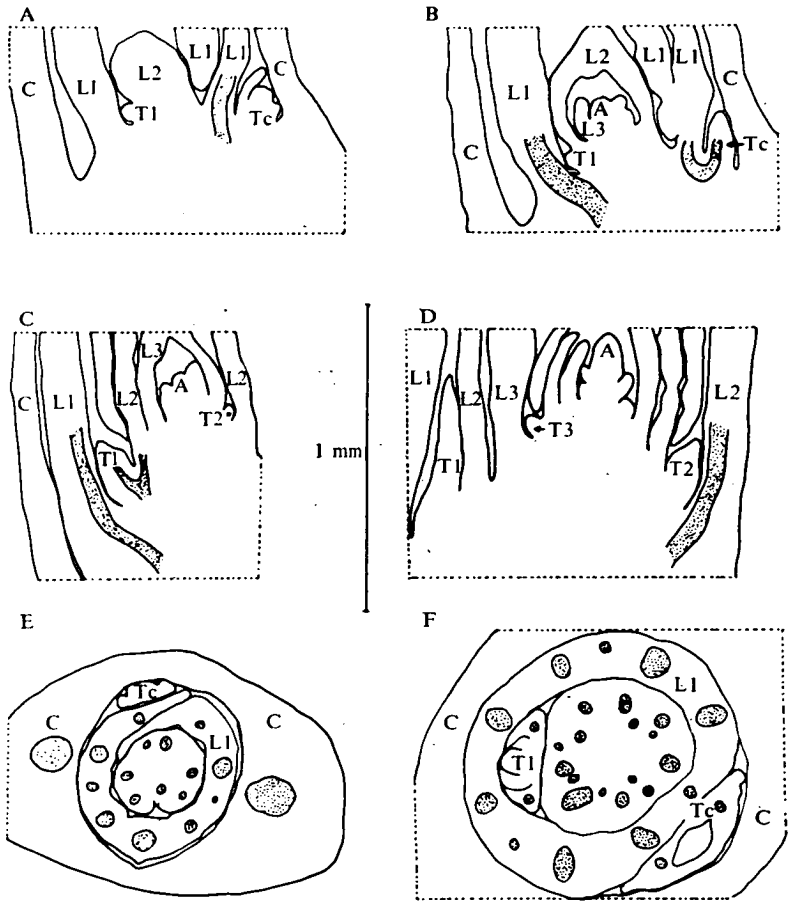

FIG. 1. Camera lucida drawings of longitudinal (A–D) and transverse (E, F) sections of the apical regions of young barley seedlings.
L = leaf, T = tiller, A = main-stem apex. Stippled area indicates visible vascular strands.
A, B show material 2 days old.
C, F show material 6 days old.
D shows material 10 days old.
E shows material 1 day old.

six and seven leaf primordia were present on the main stem. It may be concluded therefore, that up to day 10 new main-stem tiller buds were initiated every 4 days or so. This is slightly longer than the foliar plastochron (Table 2, see also Dale *et al.*, 1972). As the apical region came to include more leaves, with resultant close association of these structures, the unequivocal identification of the very small bud initials became more difficult and no studies were carried out beyond day 10.

Between days 1 and 10 Tc developed from a well-defined bud with a prophyll to a structure with prophyll and three leaf primordia. In the same period T1 developed from

being hardly visible to a structure also having a prophyll and three leaf primordia. Thus T1 appeared to develop more rapidly than Tc although the latter was still larger than the T1 bud at day 10.

Vascular connections to tiller buds were investigated using serial sections of the apical region; Fig. 1 comprises camera lucida tracings of sections chosen to show relevant features.

The first signs of a vascular trace within Tc were visible on day 2 and a connection to a trace leaving the first leaf is apparent (Fig. 1B). In other sections, two traces supply the bud and these run laterally along the flanks of the somewhat flattened structure (Fig. 1F). The coleoptile traces are found some distance from the Tc bud and it is with traces at the edge of the first leaf, L1, that the Tc traces are connected (Fig. 1E, F). Lignified tissue was not seen in Tc until days 7–8.

TABLE 2. *Appearance of tiller buds and numbers of leaves on mainstem and tiller buds on seedlings of* Proctor *Barley up to day 10*

	Age of plant	Mainstem	Tc	T1	T2	T3
Set 1	24 h	C+4	P			
	48	C+4	P+1	*		
	72	C+5	P+1	P		
	96	C+5	P+1	P		
Set 2	4 days	C+5	P+1	P		
	6	C+6	P+2	P+1	*	
	8	C+6	P+3	P+2	P	
	10	C+7	P+3	P+3	P+1	*

C = coleoptile, P = prophyll.
* = Tiller present without defined prophyll.

T1 and subsequently formed buds are initiated in close association with the base of the next-highest leaf (e.g. Fig. 1A–D). Traces in T1 were not readily visible until about day 6 at which time connections with lateral traces in L2 were also apparent (Fig. 1C). However, sections of younger material show that a substantial trace (or traces) from L1 passes close to the base of T1, even as early as day 2 (Fig. 1B; also seen at a later stage in Fig. 1C).

Vascular traces to the T2 bud were visible by day 10, about 4 days after bud initiation. These traces eventually link with L3, although as for T1 the bud is close to strands running out of the subtending leaf (Fig. 1D).

Summarizing, analysis confirms that vascular connections occur between the tiller bud and the leaf above it (Bunting and Drennan, 1966; Langer, 1972). During the early developmental stages there appears to be no direct link between traces in the bud and subtending leaf. Tc can be distinguished from T1 and T2 since it develops distally from the traces of the subtending coleoptile whereas the later-formed tillers show a close, but not direct, association with the subtending leaves. It is also apparent from many dissections that area of contact between the base of Tc and the adjacent main stem is less than that for T1; in consequence Tc appears to be a narrower structure and is more easily broken from the stem during dissection.

Early growth and the effects of leaf shade treatments on tiller buds

Because of the physical association between tiller buds and main-stem leaves a comparison of growth in dry weight of these organs was of interest (Fig. 2). This showed that early growth of primordia L2, L3, L4, and L5 was similar and exponential up to time

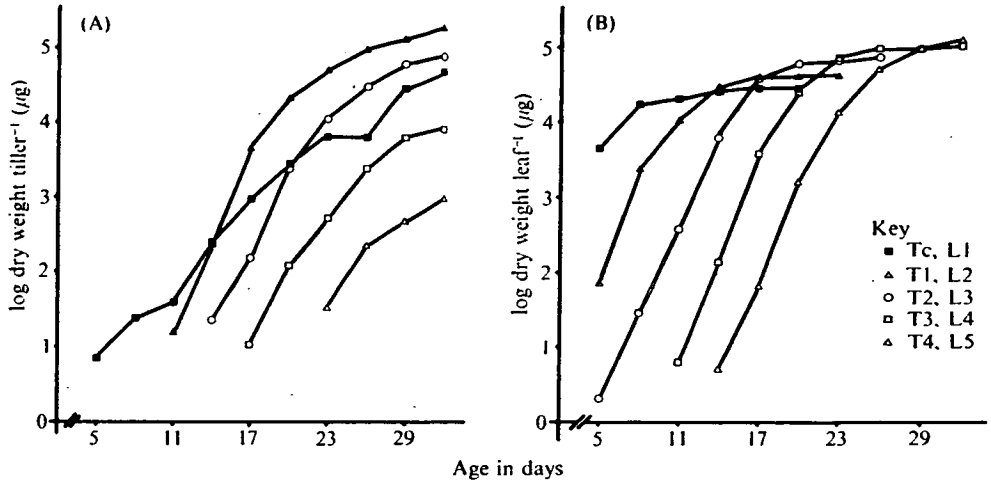

FIG. 2. Time course of growth in dry weight of successive primary tillers and main-stem leaves.

TABLE 3. *Maximum mean relative growth rates (g g^{-1} day^{-1}) for tillers and leaves. The 3-day period over which the value was obtained is shown in parentheses*

Tc	T1	T2	T3	T4
0·60 (11–14)	0·99 (14–17)	0·92 (17–20)	0·74 (17–20)	0·65 (23–26)

	L2	L3	L4	L5
	1·17 (5–8)	0·94 (11–14)	1·12 (14–17)	1·06 (17–20)

of appearance and unfolding when relative growth rate began to decline. A period of exponential increase in bud dry weight was also found. T1, T2, and T3 showed more rapid initial growth rates than Tc, confirming the impressions derived from anatomical examinations. Maximum growth rates of tiller buds were consistently less than those of the associated leaves and were reached later (Table 3).

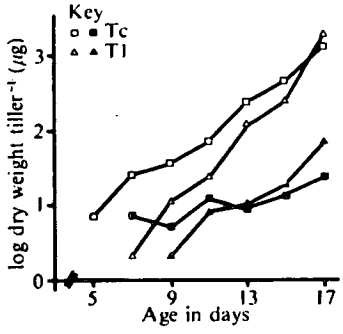

FIG. 3. Growth of Tc and T1 tillers grown under control conditions (open symbols) or with the first leaf shaded (closed symbols).

Shading the first leaf, thereby eliminating its contribution to photosynthesis, is known to affect growth of the second and subsequently formed leaves (Felippe and Dale, 1973) and also to reduce the frequency of appearance of the Tc tiller (Dale *et al.*, 1972). It was therefore expected that shade would affect growth of the buds and this was found for Tc and T1 (Fig. 3).

For unshaded plants growth of both buds was exponential up to day 17. The relative rate of growth of Tc, measured as the slope of the linear regression for the data, was again significantly less ($p = 0{\cdot}05$) than that of T1. By day 16 both buds were similar in dry weight and emerged between days 19 and 22.

Examination of data for the control plants showed that the coefficient of variation for weights of Tc increased steadily with age, from 3·7 per cent on day 7 to 21·4 per cent on day 17. This was associated with a tendency for the data to fall into two classes, one showing low values, the other showing high bud weights. The calculated slope of Tc which is based on mean weights will therefore overestimate growth for some buds and underestimate it for others. The significance of this effect which has been regularly observed for Tc is discussed later; there was no evidence for changes in the coefficient of variation of weights of T1.

Growth of Tc and T1 in shaded plants was slight up to day 13 and at this stage the buds weighed less than a tenth of those in the control sets. After day 13, growth rate of the buds rose significantly, that for T1 being greater than that for Tc. In shaded material the second leaf is large and very active photosynthetically at this stage (Dale and Felippe, 1972) and the more rapid growth of buds could result from an increasing supply of assimilated material from that leaf.

Mineral nutrition and tiller bud growth

The shade experiment suggested the importance of assimilates produced by the first leaf for bud growth and knowing the effects of time of application of nitrogen on photosynthesis in the first leaf (Dale, 1972) it was thought that delay in supplying mineral nutrients, particularly nitrogen, would also affect bud growth. Experiments testing this involved varying time of application of all the mineral nutrients, the nitrogen source only, or the non-nitrogenous minerals. (See Table 1.)

The effects of delaying application of complete mineral nutrient solution are shown in Fig. 4. As was previously found, tiller buds showed a phase of exponential growth but the onset of this was determined by the timing of nutrient application. This was especially clear for Tc where little increase in dry weight above an initial value of 4–7 μg occurred until after nutrient solution was supplied; the response to treatment was detectable within 2 days. For T1 slight growth occurred in the absence of added nutrient, but rate of growth increased sharply after nutrient solution was supplied.

Linear regressions were fitted using the data for the harvest prior to nutrient application and all subsequent values (Fig. 4C and D). Comparison of the slopes showed that there was a progressive decrease in growth rate for Tc as nutrient application was delayed; growth rates for the day 8, 10, and 12 treatments were significantly less than that for the day 4 treatment, with the other values not differing from the day 4 values. A second feature was that growth rates of T1 for the day 2, 4, 6, and 8 treatments did not differ significantly but the values for day 10 and 12 treatments were significantly less than the day 4 values. It was also clear that Tc was influenced by treatment to a greater extent than T1 since both onset of exponential growth and value of the exponent were affected throughout.

Treatments also affected plant dry weight. By day 21 values for this were proportional to the time of onset of nutrient application, day 6 treatment giving a plant more than twice as heavy as treatment on day 12. Comparison of relative growth rates (Table 4) showed values for the plant to be consistently and substantially lower than those for the buds with those for Tc being invariably less than those for T1. Delay in application of nutrients from day 6 to day 12 caused a drop of 0·04 units for the plant and drops of 0·15 and 0·24 units for Tc and T1, indicating that the plant was less affected by treatment than the buds.

Since delay in the provision of non-nitrogenous mineral nutrients has negligible effects on early growth of the plant whereas delay in application of nitrogen has substantial effects (Dale, 1972) it was thought that the results of this experiment could be interpreted in terms of nitrogen supply. However, a preliminary experiment indicated a different situation for tiller buds with evidence for an interaction whereby response to added

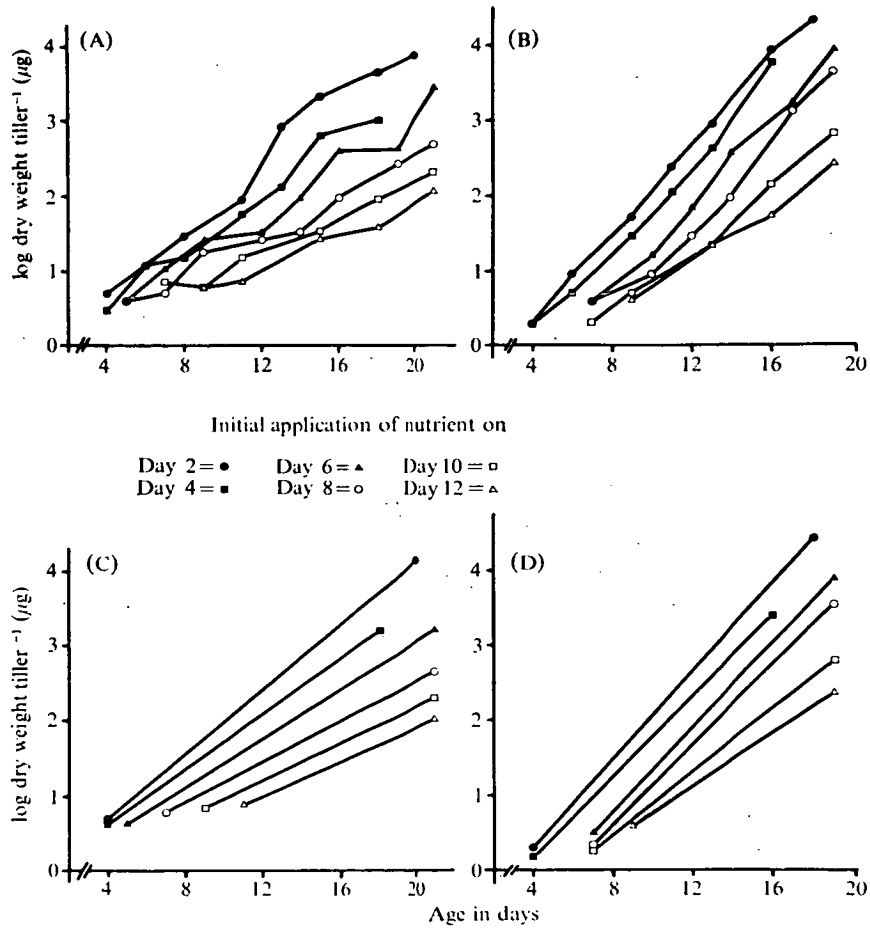

FIG. 4. The effect of delaying application of nutrient solution from day 2 to day 12 on growth in dry weight of tillers Tc (A, C) and T1 (B, D). Raw data are shown in A and B, and linear regressions on these data in C and D.

TABLE 4. *The effect of delay in nutrient application on mean relative growth rates (g g^{-1} day^{-1}) of tillers, Tc and T1, and the whole plant. Period (days) over which R was measured is shown in parentheses*

Day of initial nutrient application	Tc	T1	Plant
2	0·49 (4–20)	0·67 (6–20)	..
4	0·42 (4–18)	0·62 (6–18)	..
6	0·37 (5–21)	0·65 (9–21)	0·15 (9–21)
8	0·31 (7–21)	0·62 (9–21)	0·13 (9–21)
10	0·29 (9–21)	0·48 (9–21)	0·10 (9–21)
12	0·27 (11–21)	0·41 (11–21)	0·11 (11–21)

nitrogen was dependent on the presence of the other mineral nutrients. A detailed examination of this response was therefore carried out, in the first instance varying time of application of nitrate or ammonium as nitrogen source. Toxicity effects of ammonium on cereals are well known and unpublished experiments confirmed that *Proctor* barley

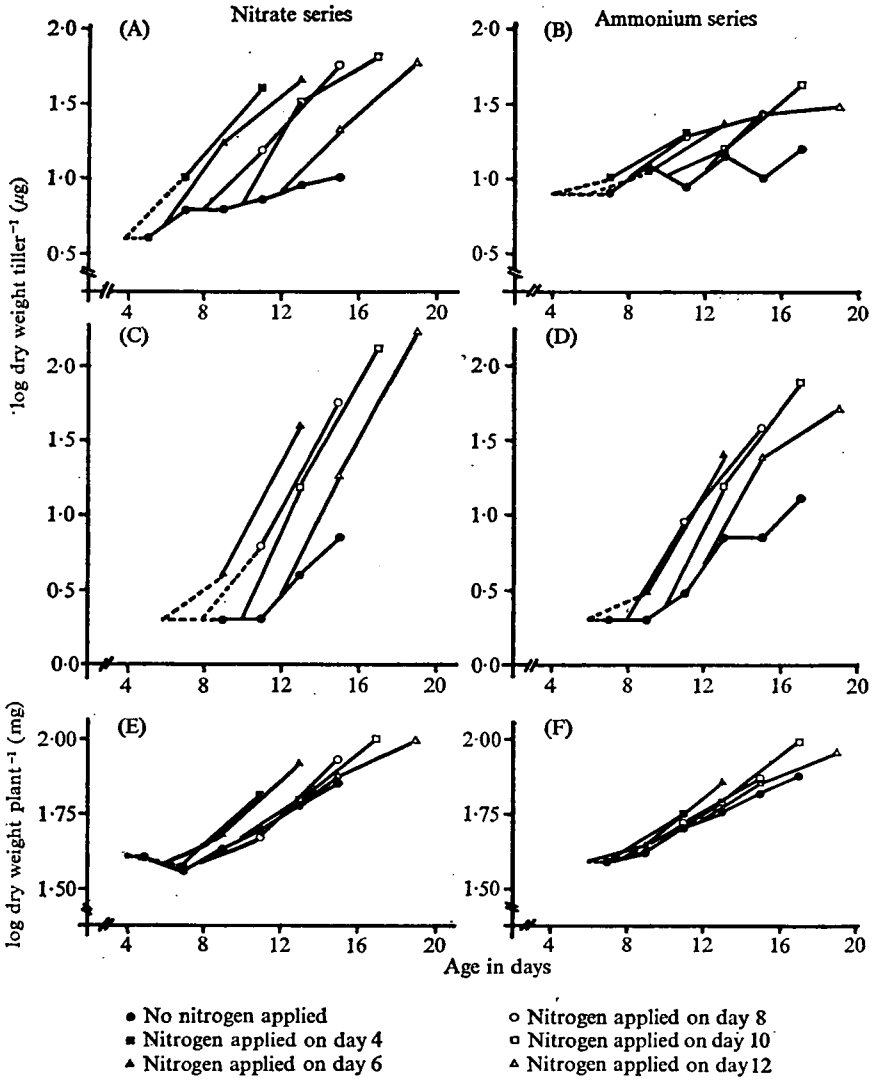

FIG. 5. The effect of delaying application of nitrogen as nitrate or as ammonium on growth in dry weight of tillers Tc (A, B), T1 (C, D), and whole plant (E, F).

grew less well supplied with ammonium. Plant and tiller bud growth was therefore determined only over a short 7-day period following application of nitrogen (Fig. 5). Little growth of Tc occurred in the absence of either source of added nitrogen, but addition of either nitrate or ammonium was followed by substantial growth of the bud by the time of the first harvest (i.e. within 3 days). The response to ammonium was less marked than that to nitrate.

Some growth of T1 occurred in the absence of added nitrogen after days 9 or 10; this contrasted with a much lower rate of growth found for the Tc controls. More rapid growth of T1 followed application of nitrogen, the response to nitrate being again greater than that to ammonium. For both Tc and T1 relative growth rates (Table 5) were broadly similar for treatments involving the same nitrogen source, with rates for T1 greater than those for Tc. Data for plant dry weight (Fig. 5E and F) indicate that nitrogen

TABLE 5. *Mean relative growth rates (g g^{-1} day^{-1}) of tillers and whole plant over the period 3–7 days after application of nitrate. Results from an experiment in which ammonium was applied in place of nitrate are shown in parentheses*

Day of nitrogen application	Days over which R measured	Tc	T1	Plant
4	7–11	0·35 (0·18)	..	0·14 (0·09)
6	9–13	0·25 (0·19)	0·57 (0·53)	0·14 (0·13)
8	11–15	0·28 (0·09)	0·56 (0·37)	0·15 (0·09)
10	13–17	0·18 (0·25)	0·54 (0·39)	0·12 (0·11)
12	15–19	0·25 (0·03)	0·56 (0·20)	0·07 (0·06)
Never	7–15	0·06	..	0·08
Never	9–15	..	0·21	..

TABLE 6. *Mean relative growth rates (g g^{-1} day^{-1}) of tillers after application of non-nitrogenous minerals on days 5, 8, or 11 to plants already supplied with nitrate*

Day of nutrient application	Days over which R measured	Tc	T1
5	5–11	0·26	..
8	8–14	0·32	..
11	11–17	0·21	0·53
Never	5–17	0·10	..
Never	11–17	..	0·23

treatments also affected this parameter such that early application led to a larger plant at any particular moment in time.

The complementary experiment, with nitrate added on day 4, showed that delay in the application of the other mineral nutrients allowed only slight growth of Tc and T1 buds, although growth of the whole plant was unaffected. Addition of the nutrients gave responses similar to those found previously in that there was considerable growth of both buds (Fig. 6). The relative growth rates were close to those found after nitrate application in the previous experiment (cf. Tables 5 and 6). However, growth of Tc was somewhat higher when nitrogen was present without the mineral nutrients than in the reverse situation (cf. baselines in Figs. 5 and 6).

Further experiments indicated a differential response of plant and buds to variations in the amount of nutrient supplied. Keeping supply of other minerals constant but varying the amount of nitrogen, supplied as nitrate, from 14 down to 0·7 mg per application led to substantial reductions in growth of plant and buds (Fig. 7). Analysis of variance showed that growth of the plant was not significantly increased when nitrogen level was raised beyond 4·2 mg $application^{-1}$. However, a level of nitrogen of 7·0 mg $application^{-1}$ had to be provided to allow maximum growth of the T1 and T2 buds. The relationship between growth of Tc and amount of supplied nitrogen is less clear. This is because

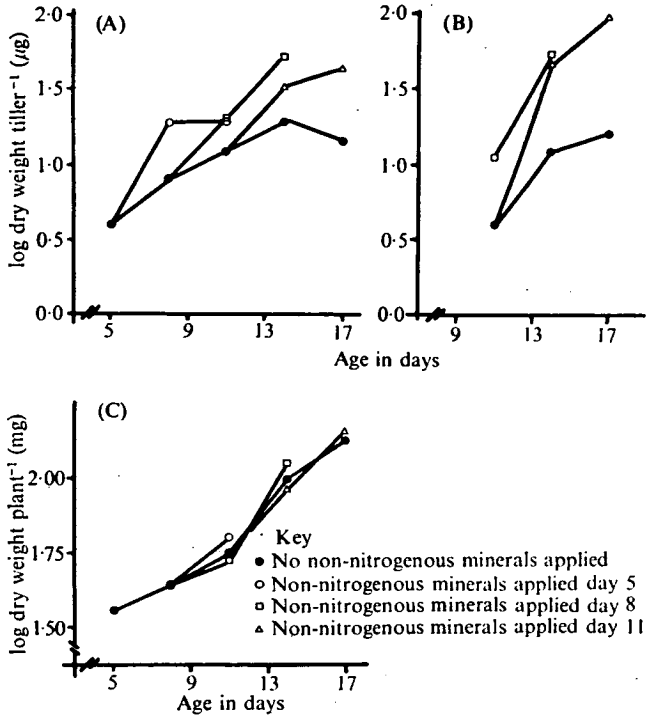

FIG. 6. The effect of delaying application of non-nitrogenous minerals on growth in dry weight of tillers Tc (A), T1 (B), and whole plant (C).

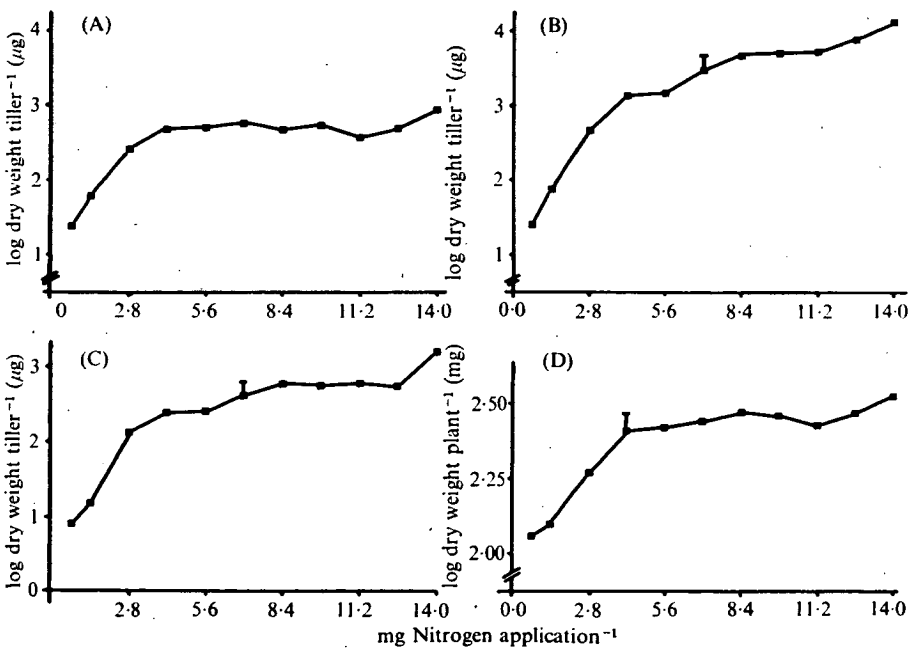

FIG. 7. The effect of increasing nitrogen supply on dry weight of tillers Tc (A), T1 (B), T2 (C), and whole plant (D), harvested on day 20. Vertical bars indicate least significant differences ($p = 0.05$) computed from analysis of variance on the data.

the tendency of the data to show bimodality (see p. 69) could not be corrected by the logarithmic transformation routinely used thus rendering the analysis of variance suspect.

Consistently high values were found for all parameters of the eight replicates given nitrogen at 14·0 mg application^{-1}. Comparison with other results makes it likely that differences between these values and the plateau values reached with lower levels of nitrogen supply are probably due to chance.

Lowering the amount of non-nitrogenous minerals, keeping nitrogen supply constant, also had different effects on plant and buds. Table 7 shows data for part of an experiment in which nitrate was supplied on day 4 and the other mineral nutrients supplied on day 11 at full or one-fifth strength. Plant weight was only slightly affected by treatment, but the buds were significantly smaller, the effect on T1 being greater than that on Tc.

TABLE 7. *The effect of supplying nutrients other than nitrogen at either full or $\frac{1}{5}$ strength on log dry weight of tillers (μg) and the whole plant (mg)*

	Tc		T1		Plant	
Solution strength	Full	1/5	Full	1/5	Full	1/5
Day measured						
14	1·51	1·23	1·67	0·95	1·96	1·97
17	1·63	1·34	1·98	1·23	2·15	2·04

DISCUSSION

Anatomical evidence and dry weight data suggest that growth of tiller buds of barley occurs in two phases. There is firstly an initiation phase, beginning with the formation of the bud primordium which goes on to produce a prophyll and up to two foliar primordia. At first there are no vascular connections to the bud but the close proximity of the central strands of the subtending foliar primordium to the bud (for T1, and subsequent tillers) suggests that a nutritional association between the two exists, whereby some of the metabolites moving to the leaf may be diverted to the tiller. Towards the end of this phase, vascular strands connecting the bud to the peripheral traces of the leaf above are developed. This indicates the onset of the second, post-initiation, phase which ends with tiller emergence. This phase is interpreted as one in which the bud is nutritionally dependent principally, but perhaps not entirely, on the leaf above the subtending leaf. The data show that the phase can under certain conditions (e.g. shade treatment, delayed mineral supply) be separated from the first by a lag period, but once it is established growth is initially exponential. The establishment of vascular strands to the bud before the onset of the second phase seems significant in allowing a more rapid and controlled flow of metabolites to the bud, even though the course of the connections suggests that supply may be monitored by the associated leaf.

The subordinate status of buds in the tiller:leaf association is indicated by three facts, the slower initiation of tiller buds which have a longer plastochron than leaf primordia, the pattern of vascular connections, and the lower maximum growth rates of buds, reached later than for the associated leaves. Together these facts suggest that leaves and buds are not in competition with each other for available metabolites but rather that bud growth is subject to control by the leaves and particularly by the leaf above the subtending leaf. The results of shade experiments support this idea. There is good evidence that shading L1 delays onset of the second phase of growth of Tc; this may be a permanent effect as a reduction in the number of emerging Tc tillers following shading has been shown (Dale *et al.*, 1972). Growth of T1 is also affected initially when L1 is shaded. The slight delay in development of L2 resulting from shade treatment (Felippe and Dale, 1973) is followed by high rates of photosynthetic activity in this leaf (Dale and Felippe, 1972)

and by rapid growth of T1. The relationship between activity of the leaf and growth of the bud is regarded as further evidence for the close association between L2 and T1 indicated on anatomical grounds.

Differences in vigour between coleoptile and first leaf tillers have been observed by many workers (Cannell, 1969; Jewiss, 1972; Kirby and Faris, 1972) and an explanation for this can be advanced. Tc is interpreted as being at a disadvantage nutritionally in both phases of bud growth compared with T1. In the first, it is associated with the coleoptile which completes growth by day 3 and presumably ceases to import significant quantities of metabolites after that time. Also the coleoptile vascular strands run some way from Tc, so that the distance over which diffusion of metabolites has to occur to reach the bud is greater. In the second phase of growth Tc is associated with L1 which is for a time the sole source of assimilated carbon for the whole of the plant including other terminal apices (Felippe and Dale, 1973). Demands from these growing points may be expected to compete with that of the bud. The segregation of coleoptile tillers into two populations, rapidly or more slowly growing, which begins to appear coincident with dependence on the first leaf suggests that metabolite supply from that leaf is not always adequate for bud growth. In contrast, T1 is initially associated with the rapidly growing L1 at a time when it imports reserve material from the endosperm through vascular connections which run close to the base of the bud. Subsequently T1 is connected to L2. This leaf, larger than L1, functions for a while concurrently with it so that more assimilates may be available for bud growth in this situation. Similar arguments can be applied in the case of T2, and indeed for all subsequently formed primary tillers, and it is of interest that the time course for growth of T1 and T2 is similar.

Turning now to effects of mineral nutrition, the data confirm that as well as affecting tiller number treatments also affect the growth of buds. In fact, in the absence of an exogenous supply of minerals tiller development does not occur. It is also clear that delay in supplying complete mineral solution has greater effects on bud growth rate than on that of the whole plant. The results for the experiment investigating concentration effects indicate that growth of the plant is not limited with a nitrogen supply substantially less than that necessary to allow maximum bud growth. In view of the small absolute requirement of the bud for nutrients, this suggests a restriction on movement of nitrogen to the bud which can only be overcome when high concentrations are supplied. Since similar results have been obtained in experiments in which concentration of non-nitrogenous minerals were varied it is concluded that there may be an accumulation of minerals in the leaf which by filtering action restricts flow to the buds. It may be significant that very high levels of nitrate have been found in the first leaf (Dale, Felippe, and Christie, in preparation).

The demonstration that a response of tiller buds to nitrogen depends upon supply of other minerals and vice versa was unexpected. Work showing the effects of nitrogen supply on tiller numbers in barley has already been quoted. More recently, McIntyre (1972) has suggested the controlling effects of nitrogen supply on bud growth in *Agropyron*. Together with the published data, the observations on the effect of shade on bud growth and the known effect of nitrogen supply on photosynthetic activity of the first leaf (Dale, 1972) all indicated that nitrogen could be the key nutrient controlling bud development, perhaps through effects on the availability of carbon assimilates. This view is no longer tenable without modification since evidence for the interaction between nitrogen and the other mineral components is unequivocal.

It may be asked which of the non-nitrogenous minerals are important for bud growth. Gregory and Sen (1937) showed effects of both potassium and phosphorus on the number of tillers produced by barley and other data have shown the importance of phosphorus for bud growth (Fletcher, unpublished). But the key question concerns the roles of the necessary nutrients and a number of possible explanations can be advanced. It seems

plausible to argue that nitrogen is of more fundamental importance than the other minerals. The reasons for this view are firstly that endogenous nitrogen is insufficient to allow maximum development of the first leaf (Dale, 1972) which it is argued controls flow of metabolites to Tc and T1; secondly, that nitrogen will be required in substantially larger amounts than other minerals in growing buds, as in any other growing tissues; and thirdly, that a small response of buds to added nitrogen is found in the absence of other nutrients but not vice versa. If the requirement of the bud is primarily for assimilated nitrogen produced in the leaf then the role of the other minerals could be in the operation of a clutch mechanism facilitating entry or incorporation of organic nitrogen into the bud so allowing growth. Delay in application of complete mineral solution reduces bud growth rate in the second phase whereas delay in nitrogen application does not (cf. Tables 4 and 5 for Tc). This could indicate that early supply of non-nitrogenous minerals is necessary to preserve the capacity of the bud for maximum growth, a capacity which is progressively reduced if supply of these minerals is delayed. Further work is proceeding to clarify details of the mechanisms involved which could be located in the bud itself or elsewhere in the plant.

The final point concerns the implication of these results for theories of apical dominance. Our interpretation is that control of growth of the tiller buds has a nutritional basis; however, the nutritional requirement is unspecific in that assimilated carbon and at least two mineral nutrients have to be supplied before growth in the second phase will occur. We know of no evidence to show that application of growth substances will trigger off tiller bud growth. It is, however, accepted that provided the basic nutritional requirements for minerals and carbon are met, addition of growth-active substances to plants may enhance or even inhibit growth as has been shown by Jewiss (1972).

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ABSTRACT OF THESIS

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Title of Thesis Experimental Studies on Tillering in Barley.

There were two main aims in this experimental project on tillering in barley; first, to obtain information on the rates of growth, and various aspects of stem development on both tillers and mainstem, and secondly, to study the early growth of tillers prior to their emergence above their subtending leaf sheaths, and the effects of various treatments on this.

Shading either the first or second mainstem leaf was found to reduce the numbers of tillers emerging and to decrease total plant grain yield without affecting yield of either the mainstem or individual tillers developing to maturity; the major effect of shading either leaf was shown to be on tiller development during early plant growth. Rates of leaf appearance on the mainstem and tillers developing to maturity were not affected by shading, and were found to be in the order - mainstem primary tillers secondary tillers. A similar order for the different stems was found when rates of primordial initiation were studied. These results indicate a hierarchical relationship between stems in the barley plant.

Both the onset and the rate of exponential increase in dry weight were also affected when the initial application of the complete mineral nutrient solution was delayed beyond day 2 after planting; there were also considerable effects of delaying the second application of nutrient. Withholding either nitrogen or non-nitrogenous nutrients, and lowering the amounts of nitrogen applied seriously affected tiller bud growth, and it was shown that the effects of lack of non-nitrogenous nutrients were due primarily to the lack of phosphorus. TC was affected to a greater extent than T1 when nutrient application was delayed, but the reverse situation was found when amounts of nutrient applied were decreased. Tillers were consistently affected to a greater extent than the plant by delaying the application or decreasing the amounts of nutrients applied, indicating an effect of nutrient application on apical dominance.

Studies of tiller bud development in control conditions were also carried out; both initiation of floral primordia, and awn appearance took place later on the the tillers than on the mainstem, and both these developmental stages were found to be more or less synchronous on tillers/

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tillers T1 - T3. Early in plant growth the mainstem apical dome was larger than the corresponding region on any of the tiller apices, although after floral initiation apical dome size was similar on both mainstem and tillers T1 and T2. Relationships between young buds and the mainstem were investigated by both a ^{14}C-labelling experiment, and an anatomical study of sectioned material from seedlings up to 10 days old.

From the results of the project possible routes for the passage of assimilates and mineral nutrients into tiller buds during their early development are suggested, and the implications of the results on current theories of apical dominance, and the possible importance of the commercial use of uniculm cereal varieties discussed.